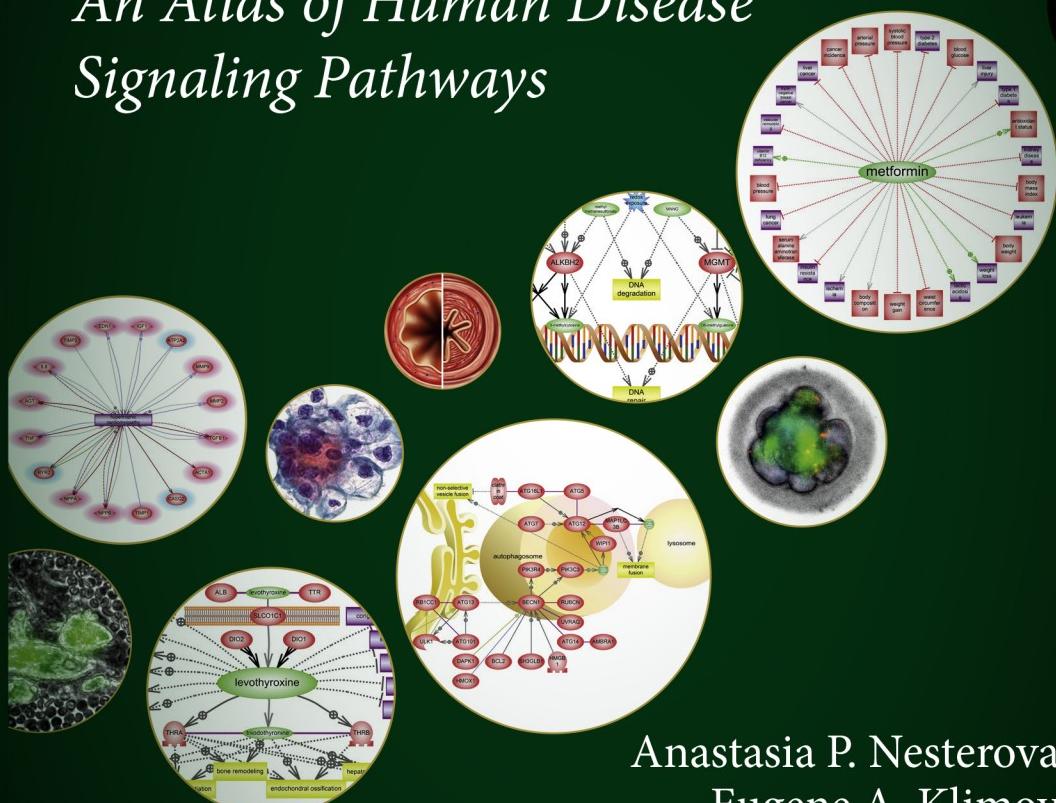


# DISEASE PATHWAYS

*An Atlas of Human Disease  
Signaling Pathways*



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## DISEASE PATHWAYS

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# DISEASE PATHWAYS

## An Atlas of Human Disease Signaling Pathways

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#### Library of Congress Cataloging-in-Publication Data

A catalog record for this book is available from the Library of Congress

#### British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

ISBN: 978-0-12-817086-1

For information on all Elsevier publications  
visit our website at <https://www.elsevier.com/books-and-journals>

Publisher: Stacy Masucci  
Acquisition Editor: Tari K. Broderick  
Editorial Project Manager: Anna Dubnow  
Production Project Manager: Maria Bernard  
Cover Designer: Matthew Limbert

Typeset by SPi Global, India



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# Contributors

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Many thanks to Maria Shkrob, PhD, and Anton Yuryev, PhD, for their broad advice and for participating in constructive discussions. Maria and Anton work on the front line of biomedicine and pharmacological research in Elsevier Life Science Services and have diverse skills and experience. Maria and Anton are experts in natural language processing and term taxonomies. Dr. Anton Yuryev is the leading specialist in the area of personalized medicine and the pathway analysis of biomedical experiments.

Special thanks to Paul Golovatenko-Abramov, Andrey Kalinin, Philipp Anokhin, Chris Cheadle, Stephen Sharp, and the rest of the team of Pathway Studio for their advices and technical support.

# Foreword: The future of medical discovery

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For centuries, scientists and clinicians have published their findings in the traditional professional literature. Many of us, as scientists, clinicians, or as students of science or medicine, have read that the literature voraciously trying to understand the complexities of biological systems. Perhaps the most challenging systems are those of humans because, for a variety of good reasons, we cannot perform laboratory-based studies on humans. Instead, we turn to model organisms or the results of clinical observations.

Our lack of understanding arises from both the challenges of ingesting the sizeable corpus of literature and the natural and inescapable variations observed in biological systems. This book coalesces a wealth of scientific and medical literature into an organized body that is rich with illustrations and explanations. As an atlas of selected human disorders, the facts regarding involved entities and their relationships depicted herein are the products of computer-based analyses of published literature. Those analyses exploit the tools of natural language processing applied to existing literature along with the expertise of a group of scientists with extensive and complementary experience. The resulting disease pathways were created by those scientists based on the entities and relationships revealed through their computer-based analyses.

This work is remarkable in several regards. It is the product of the collaboration between an amazing technology and human curation. Currently, there are a variety of software tools that enable us to explore, mine, and reduce an otherwise unapproachably large and complex body of literature to identify relationships among molecular entities such as genes and proteins, cells of similar and disparate types, tissues and organ systems, and, in some cases, environmental factors and deduce their roles

in human disease. That alone is a feat that was impossible only a few years ago. In this work the authors provide information about a number of human diseases by walking us through the fruits of their analyses of several infectious diseases and many human physiological systems. In that regard, this book is an outstanding example of contemporary computational systems biology.

Another unique aspect of this work is the team of scientists who brought their collective expertise to bear on the challenge of understanding and assembling the results of those computerized analyses. Nature is inescapably interdisciplinary and interdisciplinary teams that are necessary to truly understand it. Consequently, this book is the result of collaborative efforts among natural and medical scientists.

Finally, this book embodies and foretells the future of medical discovery. The vast and rapidly growing body of scientific and medical literature can only be understood by using computerized tools joined with the expertise of scientists and clinicians. Computers can identify entities and relationships involved with disease, but humans must apply their knowledge to verify those results. This collaboration between computers and humans is likely the only way that medical discovery can proceed at a rate that will ensure the deep understanding of disease, at the molecular level, that will be required for the development of effective diagnostic and therapeutic techniques.

# Preface

---

The size and volume of our knowledge are replenished very quickly in the modern era of biology. Every week, new facts are published, new genomes are sequenced, and new proteins are discovered. Unlike any time before now, a detailed understanding of the behavior of biomolecules, changes in gene expression, the regulation of hormone synthesis, or enzymatic reactions are available for the diagnosis and pharmacological manipulation of diseases.

This book is designed to fill a void in the literature of illustrated reviews of the well-documented and understood mechanisms of human disease at the cellular and molecular levels. The core of the book is based on the Elsevier Disease Pathway Collection (signaling pathways of 250 diseases), which was compiled by the team of authors throughout 2012–18 (<http://www.pathwaystudio.com>).

Pathways and networks are becoming indispensable tools in many areas of molecular biology, pharmacology, and medicine. Our knowledge about protein-protein interactions accumulated in the form of pathway models allows us to interpret the results of molecular screenings and identify targets for drug design. A unique aspect of this book is the images of disease pathways, which are a registered trademark of Elsevier, and this is the first time they are being published outside of Elsevier's commercial software. A link to the repository where the described pathway models can be freely browsed or downloaded is also provided.

The most difficult part of assembling this book was deciding which diseases to include. There are so many diseases that were covered by the team of authors and so few available pages to fill. We have included 42 the most common widespread diseases in the human population and other well-studied ones that can together illustrate the interactions between molecular causes and disease symptoms.

We have not covered oncological diseases because we believe that the oncology domain must be described separately as an intensively studied and important part of human disease. We also have not included syndromes (with rare exception) or mental disorders in this book, but we hope we can publish them in the future.

## Objectives

This atlas has a dual intent: to provide readers with detailed information about the basic concepts of disease signaling pathways on a high scientific level while simultaneously keeping the presentation simple and clearly understandable for a general biologist.

To meet these objectives, we shaped disease descriptions as an atlas, a richly illustrated book with a short narrative. We listed definitions and all terms that facilitate understanding disease mechanisms before the description of molecular signaling pathways for each disease. At the same time, we attempted to motivate readers to find more information about each disease by providing specialized web links and references.

The authors are not responsible for any conclusions or consequences arising from the use of the information presented in this book such as drug names and another disease treatment-related information. Any drugs or products mentioned in this publication should be used in accordance with the prescribing information prepared by relevant specialists or the drug's respective manufacturers.

## Assembly

This book is divided into three parts:

The first part gives introductory information about pathways as described in the guide and legend. [Chapter 1](#) in the first part provides an introduction to cell biology, molecular signaling biochemistry, and a systems biology approach to the study disease mechanisms.

The second part ([Chapters 2–13](#)) focuses on an overview of the molecular mechanisms underlying 42 diseases and covers 12 areas of human disease. Diseases are grouped into 12 areas following the International Statistical Classification of Diseases and Related Health Problems, <https://icd.who.int>. Each disease description includes a summary, a short glossary, and images of disease signaling pathways along with a descriptive narration. The mechanisms of molecular signaling are discussed briefly but thoroughly.

[Chapter 14](#), located in the third part, discusses the application of signaling pathways and networks in the analysis of big data for personalized and precision medicine. The third part also includes a glossary and the links to other resources.

## Intended audience

The book is intended for general biologists, bioinformaticists, medical workers, and students. Readers who require information about the cellular and molecular mechanisms of human diseases may also find it useful.

# Guide and legend

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## Elsevier pathway collection

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Images with disease pathways were generated with Elsevier's Pathway Studio software (<http://www.pathwaystudio.com>). Pathway Studio provides a combination of three foundations: a graph database with biological interactions extracted from millions of scientific articles using natural language processing technology (NLP) technology, a flexible visualization tool for building models of pathways, and an analytical tool for performing bioinformatics analyses of experimental results.

The Elsevier Pathway Collection, 2018 contains 2411 pathways and groups that cover 290 diseases, 6351 proteins, 2165 compounds, and 47,865 relations in total. These are manually reconstructed models in which all molecular interactions ("relations") are curated and ensured by verifying relevant sentences from their source articles.

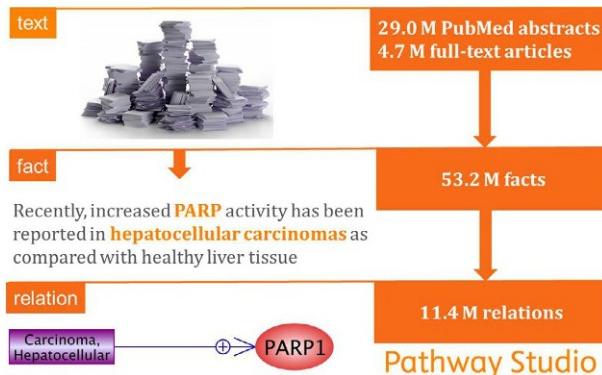
The Elsevier Pathway Collection, 2018 includes several series of pathways including biological processes, cell processes, biomarkers, receptor signaling, canonical signal transduction pathways, cell lineage, diseases, toxicity pathways, and cancer hallmarks. Unique molecular models are present in the collection such as pathways illustrating the human physiological processes of lactation and memory formation or eating behavior. For public access to selected data, please visit Ask Pathway Studio, <https://mammalcedfx.pathwaystudio.com/app/search>.

## Elsevier pathway studio database

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The Elsevier Pathway Studio database (PSD or ResNet) is the core of the system. Proteins or genes and their interactions with other biologically relevant entities are central concepts in the PSD. PSD-2018 contains 93 thousand objects and more than 11 million relationships with more than 50 million supporting facts including records from the published papers (Fig. 1). Importantly, PSD contains mammal-centered information. It covers published facts on a rat, mouse, and human molecular biology. A plant database is also available (Elsevier, 2018; Yuryev et al., 2009).

The semiautomated text-mining tool (MedScan), which uses natural language processing technology (NLP) is the basic tool for assembling the PSD. Text processing involves two critical phases: identifying concepts



**FIG. 1** Text-mining technique is a central component of the Pathway Studio.

(entities) and identifying relationships among them. The accurate and effective identification of concepts is guided by a manually created ontology of terms (names and synonyms of proteins, diseases, etc.). Moreover, MedScan identifies subject-verb-object triplets in scientific texts (e.g., insulin regulates glucose uptake) as indicators of meaningful relationships between terms (Daraselia et al., 2007; Novichkova et al., 2003). Manually written by specialists, strict linguistic rules determine the sentence structure and the type of relationship, which will be extracted. Overall, the MedScan text-mining technology has a 98% accuracy rate for concept annotation and an 88% accuracy rate for relationship extraction. Both are essentially similar to the rates achieved by expert high-quality manual annotation. MedScan can process and annotate 100,000 articles per hour allowing Pathway Studio to get updates of extracted facts and relationships weekly (Elsevier R&D Solutions, 2018).

Since PSD keeps interactions extracted from articles as object-relation type-object triplets, the union of all relationships can be visualized as one giant network or map or “knowledge graph,” and the individual pathway models are the parts of this map.

Individual disease pathway data files are written in a special Pathway Studio format (RNEF) and represent the model of interactions between molecules and concepts. RNEF files keep shaped images of members or interactions as clickable links to their descriptions and annotations. In Pathway Studio, it is possible to select a member and open the descriptive page with a list of member synonyms, ontological relations, and links to external molecular databases. If an arrow is selected, it is possible to read the supporting sentences with scientific evidence (references) directly from the publications where the relationship was described. Each disease pathway from the book can be viewed as an interactive model here: <http://www.smartbio.ai/nbs/pathways> or <http://www.transgene.ru/disease-pathways/>.

As a graphical representation, a disease pathway uses different shapes to distinguish compounds and proteins. Cell types, diseases, and processes

also have their specific shapes (Fig. 2). An arrow indicates the relationship between objects. Arrowheads graphically indicate the direction of the interaction and whether its effect is promotive (positive) or suppressive (negative) (Fig. 3). Types of interactions are marked by the color and style of arrows. For example, a gray dotted line without a head indicates an association

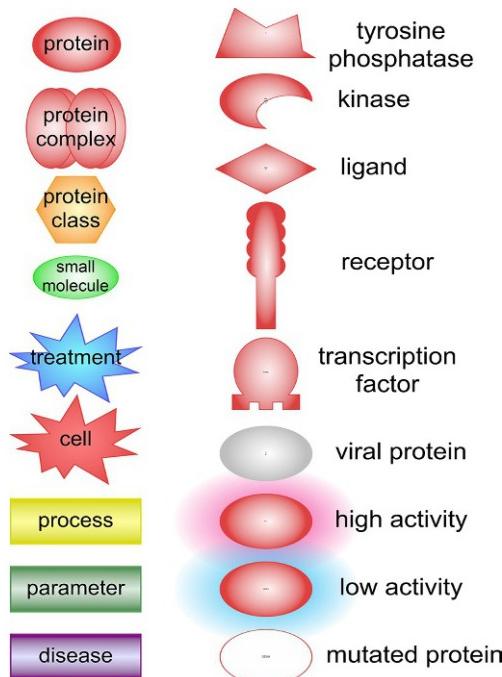


FIG. 2 Shapes of different concepts used in Pathway Studio and in the book.

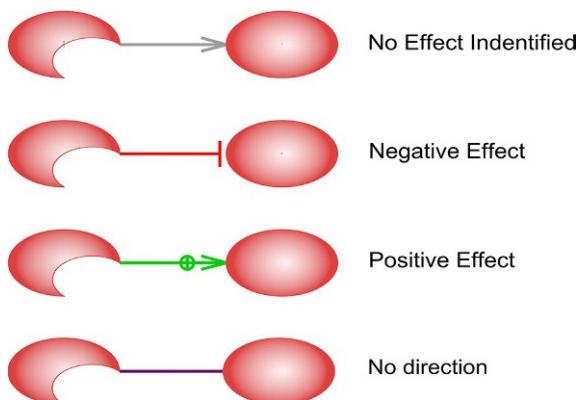
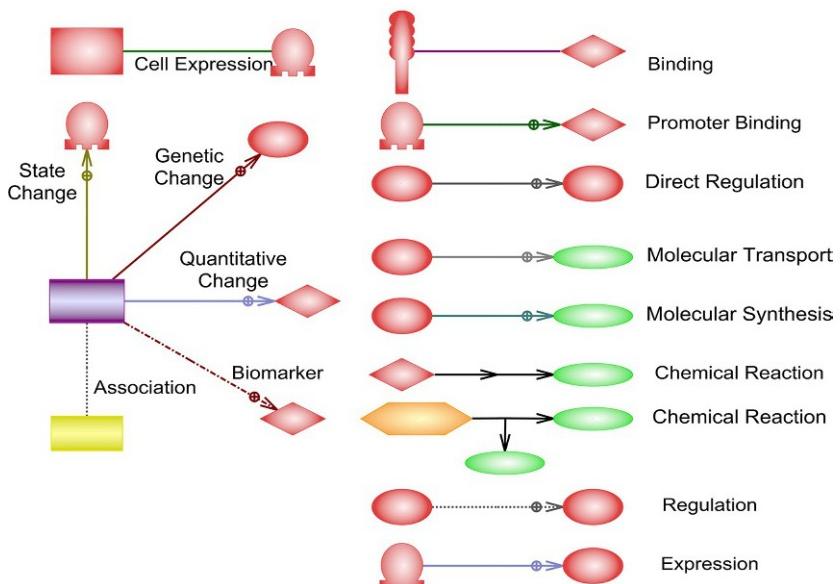


FIG. 3 Graphical representation of relationships between concepts used in Pathway Studio and in the book.



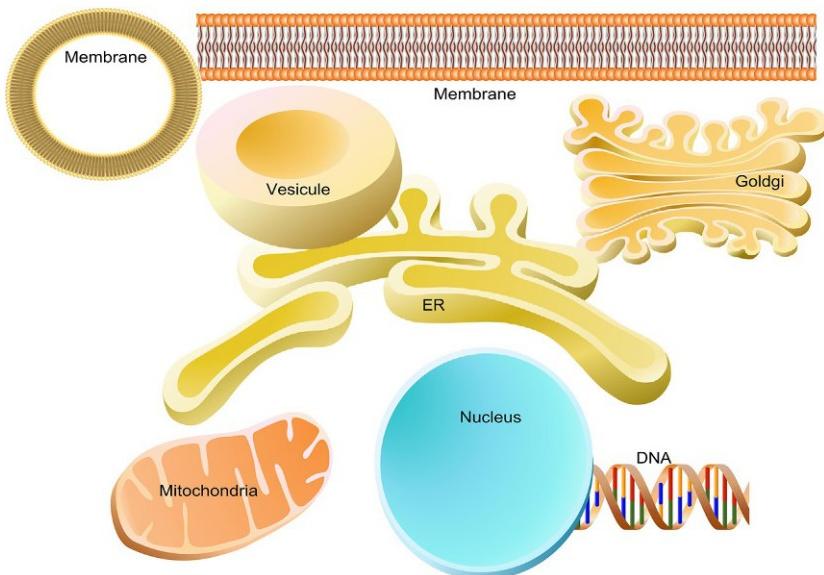
**FIG. 4** Types of relationships between concepts used in Pathway Studio and in the book. Physical connections (top); interactions between molecules (right); associative connections between a disease and other concept (left).

between two concepts or processes rather than a relationship between physical objects (Fig. 4). Although the direction and effect are the most vital pieces of information for the graphical representation of pathways, having different types of relationships is also very important for bioinformatic analyses where the pathway is used as a data file (read more in Chapter 13). Finally, stylized images illustrate intercellular components and organelles (Fig. 5).

Elsevier Pathway Studio software and the ResNet database (PSD) support and keep data about molecular interactions for at least nine types of members such as proteins, cells, or diseases and 14 types of relationships between them.

## Gene and gene products

On disease pathways and in the ResNet database, genes and gene products, including proteins, RNAs, and their isoforms, are combined in one concept type defined by the identification number of the gene in the NCBI Gene database (National Center for Biotechnology Information's, [www.ncbi.nlm.nih.gov/gene](http://www.ncbi.nlm.nih.gov/gene)). The NCBI Gene database is the gold standard for gene-centered resources (Brown et al., 2015). Therefore, different protein shapes used in the images of the disease pathway (Fig. 2) may refer to a gene, a mRNA, or a protein. There are 30,494 individual human gene concepts that have relations in the PSD-2018.



**FIG. 5** Images of cellular organelles and components used in Pathway Studio and in the book.

## Protein complexes

Several proteins can be grouped and represented as a single object in the database termed the container-type object. *Protein complex* is the first type of container object which specifies proteins that form a complex via physical interactions. For protein families or functional classes, *protein class (functional class)* is the second type of container object which groups proteins according to their classification in the molecular function gene ontology (GO) project (Ashburner et al., 2000; Gene Ontology Consortium, 2017). GO keeps biological terms organized in a hierarchical structure with protein and gene names associated with those terms. The Enzyme Commission (EC) database, BRENDA, UniProt, and other knowledge bases well known for containing protein families are also used as sources for the Pathway Studio *protein class*. There are 4513 *protein classes* and 973 *protein complexes* in the PSD-2018.

## Compounds, peptides, and small molecules

The mixed group of chemicals and metabolites is represented as a single object type *small molecule*. Members of this type are typically described and have a unique number in either the CAS or PubChem databases (<https://www.cas.org>, <https://pubchem.ncbi.nlm.nih.gov>). The *small*

*molecule* object type includes pharmaceuticals, naturally occurring metabolites, peptides, environmental, and other chemicals. There are 984,715 *small molecules* in the PSD-2018.

## Cells

The *cell* object type includes the names of different mammalian cells and cell phenotypes as well as experimental cell lines. 3824 *cells* have relationships in PSD-2018.

## Diseases

Medical Subject Headings (MeSH) from the National Library of Medicine (<http://www.nlm.nih.gov/mesh/meshhome.html>) are used for the names of health-related conditions and diseases which represent a separate object type *disease*. There are 15,640 *diseases* in the PSD-2018.

## Processes, parameters, and treatments

Events and the course of events or appearances related to human body functioning are also objects in the PSD. *Cell processes* mostly include various events occurring throughout the life cycle of a cell. The majority of *process* terms correspond to the gene ontology biological processes classification. *Clinical parameters* are measured parameters of the human body commonly used in clinical practice. Finally, the *treatment* object type accounts for nonmolecular conditions that cause changes in cellular behavior (e.g., cold shock or draft). There are 8673 *processes*, 4016 *clinical parameters*, and 65 *treatments* in the PSD-2018.

## Physical interactions between molecules

There are four different types of physical molecular interactions in the database: *Binding* is a relationship without direction because it indicates direct physical connections between biomolecules. *Promoter binding* indicates that a molecule regulates the expression of a gene by binding its promoter region. *Protein modification* is the term for a change in the molecular structure of a target protein by the direct interaction with its regulator or another molecule. Different mechanisms can be used for protein modification, such as cleavage, proteolysis, ubiquitination, acetylation or deacetylation, phosphorylation or dephosphorylation, and methylation or demethylation. *Direct regulation* depicts the physical interaction between the involved molecules in general if one of the proteins changes its activity as a result. In total, PSD contains 1,940,471 individual physical interactions between molecules.

## Indirect interactions between molecules

The situation when one molecule changes the activity of another by unknown and probably indirect mechanisms, for example, through several intermediate modifications, it is described by the *regulation* type of relationship. When the alteration of activity is known to occur due to a change in protein or RNA expression levels, the *expression* type of relationship is used. There are 5,342,470 indirect relations in the PSD-2018.

## Chemical transformations

*Molecular synthesis* and *chemical reaction* are relation types for chemical transformations. *Molecular synthesis* indicates a reaction wherein a regulator changes the concentration of molecules. The *chemical reaction* relation in PSD is used to describe enzyme-catalyzed reactions in general. There are 204,722 chemical transformation relationships in the PSD-2018.

## Molecular transport

The *molecular transport* relation type describes events involving protein and compound translocation, export, import, or release. There are 250,917 *molecular transport* relations in the PSD-2018.

## Changes in molecular activity related to other concepts

Changes in the activity levels of molecules in connection with the cell type, disease, or biological process define several relation types. Proteins expressed in a particular cell type are connected with the cell by the *cell expression* type of relation. Unspecified observations related to changes in molecular levels observed in the disease are called the *quantitative change* type relationship, while specific changes in alternative mRNA splicing or in a protein's posttranslational modification status is named *state change*. A separate relation type termed *genetic change* describes different genetic mutations associated with a disease. There are 1,929,382 of these types of relationships in the PSD-2018.

## Associations between the functions of molecules and other concepts

Associations between a disease and a cell process are shown with the nondirectional type of relation type *functional association*. *Clinical trial* is used to describe the trials conducted between drugs and diseases.

Associations between a disease or a cell and a molecule are shown as relations of *biomarker* type. In total, there are 1,735,575 associations in the PSD-2018.

## References

- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., Sherlock, G., 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29. <https://doi.org/10.1038/75556>.
- Brown, G.R., Hem, V., Katz, K.S., Ovetsky, M., Wallin, C., Ermolaeva, O., Tolstoy, I., Tatusova, T., Pruitt, K.D., Maglott, D.R., Murphy, T.D., 2015. Gene: a gene-centered information resource at NCBI. *Nucleic Acids Res.* 43, D36–D42. <https://doi.org/10.1093/nar/gku1055>.
- Daraselia, N., Yuryev, A., Egorov, S., Mazo, I., Ispolatov, I., 2007. Automatic extraction of gene ontology annotation and its correlation with clusters in protein networks. *BMC Bioinform.* 8, 243. <https://doi.org/10.1186/1471-2105-8-243>.
- Elsevier, 2018. Biological research—Pathway Studio, Elsevier, [WWW Document]. URL <https://www.elsevier.com/solutions/pathway-studio-biological-research> (accessed 12.26.18).
- Elsevier R&D Solutions, 2018. Case Study: Mining text to deliver answers on demand. Elsevier Text Min.
- Gene Ontology Consortium, 2017. Expansion of the Gene Ontology knowledgebase and resources. *PubMed, NCBI Nucleic Acids Res.* 4, D331–D338. <https://doi.org/10.1093/nar/gkw1108>.
- Novichkova, S., Egorov, S., Daraselia, N., 2003. MedScan, a natural language processing engine for MEDLINE abstracts. *Bioinforma. Oxf. Engl.* 19, 1699–1706.
- Yuryev, A., Kotelnikova, E., Daraselia, N., 2009. Ariadne's ChemEffect and Pathway Studio knowledge base. *Expert Opin. Drug Discovery* 4, 1307–1318. <https://doi.org/10.1517/17460440903413488>.

P A R T I

# Introduction

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# Introduction

## OUTLINE

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## Terminology of cellular systems

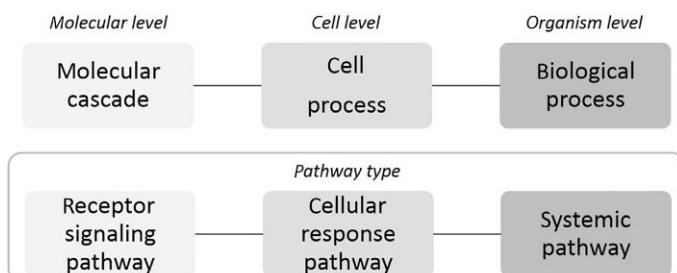
The term “pathway” represents a central theme for this book, and it comes to molecular biology from biochemistry. It is frequently used with respect to receptor signaling, described as a chain of events inside a cell triggered by interactions between an extracellular signaling molecule and a receptor on the cell surface (Berg et al., 2002; Schomburg and Michal, 2012). The very first use of the term pathway refers to the description of a metabolic reaction. A metabolic pathway diagram depicts a sequence of transformation reactions of biochemical compounds in a living organism, summing up the steps that lead to a specific product.

In systems biology, there is a need to use a broader term to describe a broader range of interactions between molecules, cellular structures, and events. There are many definitions of a pathway. Recently published reviews define biological molecular pathway as “the wiring diagrams describing the interactions of gene products and other biomolecules and their regulatory relationships corresponding to certain biological processes” (Yu et al., 2017) or as “sets of genes and/or gene products that interact with each other in a coordinated way to accomplish a given biological function” (Nguyen et al., 2018).

In this book, we define “pathway” as a chain of cause and effect links of molecular nature that connect triggers with biological processes, which can be detected by observing a distinct response.

While the starting point of the signaling pathway can be defined as binding of an extracellular molecule to the cell membrane receptor, it is not that easy to identify the final point. According to the classic biochemical description, a receptor responds to the first messengers (extracellular molecules such as hormones) and triggers the changing of properties of second messengers (such as the level of intracellular calcium), which in turn activate different cellular responses (such as apoptosis and the activation of specific genes). However, a cellular response is a multistep process itself, and it may contain cross interactions between several secondary messenger systems and several molecular events. To reduce the confusion, we suggest separating the meaning of the concept of “pathway” according to the level of organization it affects, where “systemic pathway” describes the causal chain of relations on the level of the whole organism, “cellular response pathway” describes events at the cell level, and “(receptor) signaling pathway” describes events at the molecular level (Fig. 1). Ramanan and coauthors suggested the similar principle (Ramanan et al., 2012).

A systemic pathway applies to the complex cross talk between molecular events and biological processes, describing high-level communication within an organism such as multicellular, tissue, or organ system responses. The cellular response pathway definition focuses on a chain of



**FIG. 1** Links between the pathway type and the level of biological organization.

events that involve molecular interactions inside the single cell. We will use the term “signaling pathway” to denote the classical definition of receptor signaling cascades in the cell. Receptor signaling pathway is triggered by interactions between external molecular stimuli and receptor on the cell surface.

Any type of pathway is a simplified model of the biological process on described level of organization, so many authors take this into consideration when defining the meaning and types of pathways (more on models of molecular interactions read in the succeeding text).

We will use the terms signal transduction, signaling, cascade, or circuit for intracellular blocks of the pathways, such as the MAPK signaling or the MARK cascade. However, we will consider metabolic reactions only as a part of a pathway model in this book. Although metabolic pathway has been rightly referred to as distinct model unit with the specific purpose and specifications.

There are other types of molecular interaction concepts that we believe should be separately defined. The term “network” is related to the concept of a pathway. Further, by “network,” we mean complex, nonlinear interactions of tens or hundreds of molecules and/or biological processes of any level of complexity. Also the network model can be distinguished from pathway model by the argument that networks are not vector driven from a starting point to an essential outcome (more on network model in the succeeding text). Other pathway concepts are being developed, such as adverse outcome pathway (AOP) and mode of action (MOA) models in toxicology testing. These concepts describe a cascade of key events including molecular, cellular, structural, and functional changes in biological systems that result in a measurable quantitative adverse outcome ([Bal-Price et al., 2017](#)) (more on AOP model in [Chapter 13](#) “Application of disease pathways in biology and medicine”).

With all that said, we will consistently use the following definitions that describe system interactions in an organism or a cell:

*Pathway*—a chain of causal links that connects molecular triggers and biological processes. (Examples of systemic pathway are alcoholic intoxication and hair growth. An example of signaling pathway is G protein-coupled receptor signaling, and an example of a cellular response pathway is the process of neutrophil degranulation.)

*Biological process*—a series of events with a defined beginning and end, which affects essential functions of the live organism. (Examples are nerve transmission, gastric acid release, and brain development.)

*Cell process*—a biological process that describes events on the cellular level that changes the cell’s behavior and functions. (Examples are apoptosis, chemotaxis, and phagocytosis.)

*Network*—a database of interactions between molecules or /and biological processes without clear logic and causal relationships.

## Introduction to cell signaling

All living organisms share the ability to respond to external stimuli. Cells in a multicellular organism can change their status in response to various biochemical and electrochemical impulses, and they can communicate with each other using short- and long-distance signals. The ability to receive and transmit signals is vital for survival both at the cellular and the whole organism level. Cell signaling includes three major stages: signal perception (receiving of a signal), signal transduction (composed of a series of intracellular biochemical reactions), and cellular response.

### Signal reception

Various signals stimulate the cell—molecular, electrochemical, and mechanical, or physical signals all affect and elicit a cellular response. The most common means of cellular activation is via specific proteins on the cell surface or within the cell that can sense stimulatory biomolecules. Those molecules that react to stimulatory biomolecules are termed receptors. Signaling molecules that bind receptors—termed ligands—can be proteins, peptides, or other molecules. Ligand binding causes a conformational change in the receptor and transmission of the signal to other cellular proteins, which may or may not be bound to the receptor. Intercellular interactions are also conducted through various receptor-mediated signals. For instance, the regulation of interactions between neighboring cells can involve transmembrane notch receptors that are activated by the jagged (JAG1,2) or delta (DLL) ligands to trigger evolutionarily conserved signaling pathways important for the development of tissues and organs ([Favarolo and López, 2018](#); [Polychronidou et al., 2015](#)).

The fastest mode of signal transmission is electrochemical and occurs when a change in the cell's membrane potential and ion exchange between the intracellular and extracellular environments occurs. Examples of such ions include  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$ . The transmission of electrochemical signals depends on the activation of ion channels on the cell surface. The electrochemical mode of communication is typical for neuronal and muscle cells. For example, transduction of neural impulses occurs because of the rapid generation of a  $\text{Na}^+/\text{K}^+$  ion gradient at the cell membrane, thereby altering membrane potential temporarily. The “normal” balance is quickly restored through the action of  $\text{Na}^+/\text{K}^+$  ion pumps ([Schomburg and Michal, 2012](#)).

Mechanosensitive, photosensitive, and temperature-dependent ion channels enable cells to responsive to fluctuations in light intensity, external pH, temperature, and pressure. For example, ion channels that belong to one of most studied groups—acid-sensing ion channels—respond to

changes in pH by providing a  $\text{Na}^+$  ion influx, which alters membrane potential and leads to nerve impulse transduction (Cheng et al., 2018).

Mechanical signal transduction (i.e., mechanotransduction) refers to the changing of the cell's status in response to changes in cell membrane tension. As in the case of electrochemical signal transmission, the process of converting mechanical (physical) stimuli into biochemical signals depends on the activation of ion channels. Piezo-type mechanosensitive ion channels respond to stretching of the cellular membrane by triggering a  $\text{Ca}^{2+}$  ion influx and thus activating  $\text{Ca}^{2+}$ -dependent signaling pathways (Alcaino et al., 2017).

A cell needs to respond to an enormous variety of external signals; yet, there are conserved families of receptors and ion channels that are highly similar throughout the living world. The most important and well-recognized receptor families include G protein-coupled receptors (GPCRs, or seven (pass)-transmembrane domain receptors (7TM receptors)), receptor protein-tyrosine kinases (RTKs), and nuclear receptors. Numerous reviews on the structure and function of human receptor families and studies on receptor homology among species are available (Duc et al., 2015; Lefkowitz, 2013; Moreira, 2014; Schomburg and Michal, 2012). Databases that aggregate and store information about the details of the structure and function of cell surface receptors, as well as proteins in general, are supported by large scientific institutions such as the National Center of Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov>) and the European Bioinformatics Institute (EMBL-EBI, <https://www.ebi.ac.uk/services>). These databases are publicly available.

The GPCRs, a major family of cell surface receptors in the animal kingdom, are able to bind a wide spectrum of ligands including small bioactive molecules, hormones, and neurotransmitters, as well as peptides and proteins. A typical GPCR has seven transmembrane domains and is associated with a heterotrimeric G protein complex that consists of three subunits—alpha, beta, and gamma. Each of the G protein complex subunits has several isoforms encoded by separate genes. This modularity allows for multiple combinations of subunits in a heterotrimeric G protein complex. During signal perception, ligand binding causes a conformational change in the GPCR. Guanosine diphosphate (GDP) bound to the alpha-subunit is exchanged for guanosine triphosphate (GTP), and the GTP-bound alpha-subunit then dissociates from beta- and gamma-subunits. Notably the alpha-subunit and the G protein beta-/gamma-complex activate separate paths of signal transduction in the cell (Calebiro and Godbole, 2018; Rajagopal et al., 2010; Schomburg and Michal, 2012).

Another example of a large receptor family with a vital role in cell signaling is receptor protein-tyrosine kinases (RTKs). RTKs are activated primarily by polypeptide ligands including hormones, growth

factors, and cytokines. For RTKs the mechanism of signal transmission is through receptor dimerization. Upon ligand binding the receptor dimerizes through its protein kinase domain and each subunit then transphosphorylates its dimerization partner on tyrosine residues adjacent to the kinase domain. The phosphorylated tyrosine serves as a binding site for the intracellular proteins SRC and phospholipase c gamma. SRC is encoded by a proto-oncogene and it itself a nonreceptor tyrosine kinase ([Lemmon and Schlessinger, 2010](#); [Schomburg and Michal, 2012](#); [Volinsky and Kholodenko, 2013](#)).

Nuclear receptors (receptors on the surface of the nuclear membrane) have only been discovered in animals. Their ligands are mostly lipophilic molecules and include, for example, steroids, vitamins A and D, or retinoic acid, and eicosanoids. Proteins forming nuclear receptors consist of several domains, the most important of which are the DNA-binding domain (DBD composed of two “zinc finger” sequences) and the ligand-binding domain (LBD), which sense ligands. The DBD and LBD domains are connected by a flexible “hinge” region that ensures protein trafficking inside the cell. When not activated by a ligand, nuclear receptors have the limited capability of penetrating the nucleus and acting as transcription factors ([Bridgham et al., 2010](#); [Schomburg and Michal, 2012](#)).

Ion channels are also essential for signal entry into the cell. These are pore-forming proteins and protein complexes (typically mono- and heteromers) with a major role in the ion homeostasis required for membrane potential regulation and maintaining the osmotic balance between the cytoplasm and the surrounding environment. Ion channels can be categorized as gating or voltage dependent (open in response to changes in membrane potential), ligand dependent, and others (which open in response to changes in light intensity, pressure, and temperature). Besides, ion channels can be categorized by the type of ion being exchanged. For example, there are chloride channels, potassium channels, sodium channels, calcium channels, proton channels, and nonselective cation channels. Key to the complex regulation of ion channel function is the presence of numerous feedback loops. For example, a high-voltage-dependent  $\text{Ca}^{2+}$  channel (HVGCC) opens under the conditions of altered membrane potential, allowing a  $\text{Ca}^{2+}$  influx, thereby triggering  $\text{Ca}^{2+}$  signaling. This activates protein kinase A, which in turn can phosphorylate HVGCC and alter the activity of the channel. Further details on mammalian ion channels can be found in recent reviews ([Catterall, 2010](#); [Heine et al., 2016](#); [Schomburg and Michal, 2012](#)).

There are many other proteins involved in cellular signal perception. It is worth mentioning that there are molecules involved with cell adhesion that are capable of transducing signals by interacting with intracellular proteins.

## Signal transduction inside the cell

The propagation of signals from membrane receptors and ion channels into the cytoplasm involves a host of small intracellular molecules called second messengers. Importantly, while each second messenger is responsible for activating a separate path, these paths often intersect and duplicate each other, finally leading to the activation of the same proteins. Examples of second messengers include  $\text{Ca}^{2+}$ , phosphatidylinositol 3,4,5-trisphosphates (PIP3), inositol (1,4,5), triphosphate (IP3), diacylglycerol (DAG), and cyclic adenosine monophosphate (cAMP) ([Schomburg and Michal, 2012](#)).

One of the most important secondary messengers associated with the vital activity of a cell is  $\text{Ca}^{2+}$  that gets into the cell either from the outside, through calcium ion channels, or intracellular calcium stores such as the endoplasmic reticulum. Calcium serves as a secondary messenger that participates in the activation of several  $\text{Ca}^{2+}$ -dependent signaling pathways. These include the calmodulin-dependent pathway, the phospholipase C pathway, and the protein kinase C gamma pathway. Many diseases are associated with impairment of  $\text{Ca}^{2+}$  influx or the removal of  $\text{Ca}^{2+}$  from cytoplasm, for example, familial hemiplegic migraine type 1. Excess intracellular calcium can lead to cell death.

The secondary messengers IP3 and DAG are produced in one chemical reaction from phosphatidylinositol 4,5-bisphosphate (PIP2), a reaction that is catalyzed by phospholipase C. Interestingly, DAG can activate protein kinase C, and IP3 is capable of activating specific receptors, specifically calcium channels on the membrane of the endoplasmic reticulum. This leads to activation of  $\text{Ca}^{2+}$ -dependent signaling, particularly signaling that involves calmodulin. IP3 and DAG activation are essential elements in GPCR signaling.

PIP3 synthesis requires proteins of the phosphatidylinositol 3-kinase family that can be activated by many other proteins including ones with kinase activity with the exception of G proteins. PIP3 activates the 3-phosphoinositide-dependent protein kinase that phosphorylates and activates AKT1 (i.e., the v-akt murine thymoma viral oncogene homolog 1, alternatively known as serine-threonine protein kinase B) to trigger many cellular processes. The central one leads to cellular survival by inactivating components of the apoptotic machinery.

cAMP-dependent signaling is tightly connected with G proteins and a family of proteins known as the arrestins. The former either activate (GNAS) or block (GNAI) adenylate cyclase (ADCY), a stimulator of cAMP synthesis when activated. Arrestins restrict synthesis of cAMP by blocking the G proteins. cAMP targets protein kinase A (PKA), which is a cAMP-dependent protein kinase. PKA can activate cyclic nucleotide-gated ion channels and some other proteins. The intracellular concentration of cAMP is regulated

by a feedback loop. PKA phosphorylates and activates proteins of the cAMP-dependent phosphodiesterase family, which function to hydrolyze cAMP into AMP. PKA enables such cellular processes as muscle contraction and glycogenolysis, and it stimulates some transcription factors.

cGMP is produced by the guanylate cyclase enzyme that either resides in cytoplasm where it is activated by nitric oxide or it is bound to the plasma membrane and is activated by peptide hormones. The target of cGMP is protein kinase G. The central cellular process affected by cGMP is the relaxation of smooth muscle. cGMP is also capable of activating glycogenolysis, certain ion channels, and the process of apoptosis.

## Cell response

The term “cell response” refers to changes in a cell’s status, which are experimentally observed after the cell receives a signal. Two kinds of cell response are often described, rapid, such as secretion of a product, a change in cell shape or a change in membrane potential, and slow. The latter includes the de novo synthesis, activation of transcription and translation factors, or the initiation of enzymatic cycles ([Schomburg and Michal, 2012](#)).

Changing the protein expression profile within a cell is one of the most significant cellular responses because protein synthesis defines the cellular status and its specialization and is pivotal for fundamental cellular processes. For example, these cellular processes may include the activation of programs responsible for cell division and death, adhesion, changes in cell shape, cell movement, and phagocytosis. Activation of transcription can result in the activation or suppression of protein synthesis. Suppression of protein synthesis can happen in the case of the synthesis of suppressing molecules, such as microRNAs, large noncoding RNAs (lncRNAs), and proteins that inhibit translation. Furthermore, in response to external stimuli, cells synthesize and secrete various proteins into the extracellular environment. The secreted proteins serve as mediator signals to other cells. They can be stored inside the cell in specialized vesicles and secreted on demand when a rapid response is needed. For example, granulated cells such as immune cells simultaneously release their granules with mediators in response to an external signal, typically a pathogen. This may affect the function of an entire organ or tissue.

There have been attempts to classify cellular responses. In multicellular organisms, cellular response can be divided into paracrine, intracrine, autocrine, juxtacrine, and endocrine signaling.

In paracrine signaling, the most common type of intercellular interactions, a cell reacts to an external signal by producing proteins or other molecules, which in turn serve as external signals to adjacent cells. In this case, signaling molecules are short lived and are destroyed or engulfed by

the cell, which produced them or by neighboring cells. For example, paracrine signaling is a type of intercellular communication that occurs at the synapse between the two adjacent neurons (Handly et al., 2015).

Intracrine signaling involves binding of the protein or small molecule produced in response to an external stimulus to another protein, the receptor, inside the same cell, which activates a new signaling cascade. For instance, steroid hormones synthesized and released by a cell can interact with intracellular receptors within the same cell (Re, 2003).

In the case of autocrine signaling, proteins secreted into the extracellular medium act as ligands on the same cell that produced them. The synaptic membrane of neurons, for example, often has on its surface receptors for neuromediators released by the same neurons. This allows for reciprocal regulation of the synaptic transmission of action potential (Schomburg and Michal, 2012). Autocrine regulation of tumor cells is an important factor for tumor survival and propagation as they start producing growth factors and their receptors absent in the original cell type.

In juxtacrine signaling, when a cell is stimulated by an external signal, it activates an adjacent cell through direct contact. In this case, membrane proteins of the two cells can be formally divided into "ligands" and "receptors" where the "ligands" never leave the membrane. Juxtacrine signaling is responsible for the formation of multicellular organisms; cell differentiation during embryogenesis; essential processes of the immune response, including antigen presentation; and the establishment of neural networks. For example, the membrane proteins termed integrins interact with both proteins of neighboring cells and proteins and glycoproteins of the extracellular matrix to enforce cell shape (Barczyk et al., 2010; Webb and Owen, 2004).

Finally, endocrine signaling happens when molecules produced in response to a stimulus are carried in the blood to other parts of the body where they, in turn, generate a response in distant tissues and organs. Hormone synthesis, an example of endocrine signaling, results in highly coordinated regulation of the function of the entire organism and its reactions to a changing environment (Gough, 2010; Vazquez et al., 2012).

Thus cellular responses include changes in biochemical processes, protein and RNA composition, membrane potential, physical structure and size of the cell, and the release of signaling molecules that can trigger further changes in the cell itself, in neighboring cells, or in tissues or organs at the scale of entire organism.

## Major signaling pathways

Key signaling pathways conserved across many multicellular organisms and present in different cell types have been well described in the literature. Canonical, well-known signaling pathways currently provide

the basis of our knowledge about underlying mechanisms of function of separate cells, tissues, and organs. A signaling pathway, therefore, is a simplified model of complex molecular interactions that take place inside a living cell. This terminology is clarified in the succeeding text. In live cells, signaling cascades largely interconnect so that it is hard to separate one pathway from another and it is difficult to describe the entire network of biochemical processes as little is known about some of them. However, it does make sense to try to separate distinctive pathways. Often, these distinctive pathways are the fastest and most straightforward signaling routes, which can be easily observed experimentally. Changes in the activity of proteins in the model pathway can be used as clear indicators of a cellular response to a specific signal, for example, when a drug is added to cultured cells.

Major signaling pathways include, but are not limited, to the WNT, SHH, notch pathways and the MAPK, RAS, mTOR, JAK-STAT, and NF- $\kappa$ B cascades.

MAPK signaling is activated by many incoming signals, and it plays an essential role in many cellular processes. Generally, MAPK, a mitogen-activated protein kinase, signaling cascade starts with the phosphorylation of MAP3 kinases and ends with the phosphorylation of nuclear transcription factors by MAP kinases. Signal flow in the MAPK cascade usually has only one direction: from MAP3K to MAPK. It is also known that MAP kinases of the same order do not phosphorylate each other (Kim and Choi, 2010; Pearson et al., 2001).

RAS subfamily signaling is composed of a group of signaling pathways where the central role belongs to small GTPase proteins of the RAS subfamily (e.g., HRAS, KRAS, and NRAS). Those are intracellular proteins that are activated by different incoming signals and pass the signal to other proteins involved in regulating the transcription of genes associated with growth, differentiation, and survival of a cell. These cellular processes are always impaired in tumor cells, which makes studying the RAS subfamily of proteins an important area of cancer research (Goldfinger, 2008; Rocks et al., 2006).

mTOR signaling is a group of signaling pathways featuring mTOR, mechanistic target of rapamycin kinase, serine-threonine protein kinase, and protein. This protein as part of two protein complexes, mTORC1 and mTORC2, regulates processes critical for cell survival including growth, proliferation, protein synthesis, autophagy, and the maintenance of cytoskeleton structure. mTOR signaling is actively studied in the fields of diabetes, cancer, and aging (Crino, 2015; Zeng and Chi, 2014).

JAK-STAT signaling is composed of a group of signaling pathways characterized by conservative Janus kinase (JAK) proteins. JAKs are activated by cell surface receptors that themselves dimerize after ligand binding (such as interleukins receptors), bringing different JAK molecules

physically close to each other and allowing them to transphosphorylate each other leading to activation. Activated JAK enzymes phosphorylate tyrosine residues in their receptors that in turn attracts nuclear transcription factors of the signal transducer and activator of transcription (STAT) family due to the affinity of phosphorylated tyrosine (phosphotyrosine) in JAKs to the src homology 2 (SH2) domains of the STATs. Then, JAKs phosphorylate tyrosine residues in STAT proteins, dissociating STATs from the receptors, so that STAT proteins can form homodimers. STAT homodimers in turn penetrate the nucleus and activate the transcription of multiple genes involved in the recruitment and survival of immune cells (Kiu and Nicholson, 2012; Nicolas et al., 2013).

WNT signaling is performed by a group of signaling pathways discovered in all animals. It is named after a specific ligand—proteins of the WNT, wingless-type MMTV integration site, family. WNT proteins undergo different modifications during their processing; the most important one is acylation that facilitates transport of WNT to cell membrane. WNT ligands bind to specific cell surface receptors including Frizzled (FZD) and the receptors LRP5 and LRP6 (LDL receptor-related proteins 5 and 6). Intracellular signaling of the WNT/FZD pathway can go several ways. Canonical signaling leads to stabilization of the cytoplasmic protein beta-catenin that accumulates and eventually gets into nucleus to interact with the transcription factor T-cell factor (TCF) and thus influence the expression of genes involved in the control of cell proliferation. This is especially true in multipotent cells. Noncanonical (beta-catenin independent) WNT signaling begins with the binding of WNT with the receptor-like tyrosine kinase (RYK) and the retinoic acid receptor (RAR)-related orphan receptor (ROR) proteins, which control cell polarity and play a role in morphogenesis (Chen et al., 2008; Komiya and Habas, 2008).

Hedgehog signaling is found in all animals with bilateral symmetry. It is based on interactions between the ligands sonic hedgehog (SHH), Indian hedgehog (IHH), desert hedgehog (DHH), and the receptors patched (PTCH1 and PTCH2) and smoothened (SMO). Binding of ligands to receptors leads to activation of SMO that is transported into the cytoplasm where it inhibits the SUFU protein, a negative regulator of hedgehog signaling. This in turn leads to activation of the GLI family of zinc finger 1 (GLI1) transcription factors and initiation of the transcription of genes responsible for embryonal development and cell proliferation. Impairment of Hedgehog signaling is found to promote angiogenesis and tumor metastasis (Jacob and Lum, 2007).

Nuclear factor kappa-light-chain-enhancer of activated B cell (NF- $\kappa$ B) is a protein complex of transcription factors that exists in almost all animal cells. It initiates gene transcription in response to various external stimuli including stress, cytokines, pathogens, and other dangers like reactive oxygen species and heavy metals. NF- $\kappa$ B plays a critical role in the

immune response, and its involvement in synaptic plasticity and memory was shown as well. The NF- $\kappa$ B complex consists of the proteins NFKB1, NFKB2, REL, RELA, and RELB. They should be considered together with their regulatory counterparts—NFKB inhibitor alpha (NFKBIA)—that block NFKB1 and that can be itself blocked by proteins of the inhibitor of kappa-light polypeptide gene enhancer family (IKBKB, IKBKG, IKBKE, and IKBKAP) or by the conserved helix-loop-helix ubiquitous kinase (CHUK). The canonical NF- $\kappa$ B signaling pathway implies activation of this system of dual inhibitors via MAPK signaling cascade that results in the activation of transcription of genes involved in the inflammatory response, cell growth, and the block of apoptosis. A noncanonical pathway was only discovered in cells of the immune system, and it is associated with activation of the NF- $\kappa$ B complex through the MAP3K14/CHUCK phosphorylation cascade. For example, this initiates transcription of ICAM1 and CXCL12 leading to B-cell differentiation ([Gilmore, 2006](#); [Hoffmann et al., 2006](#)).

In this chapter, we briefly define several well-studied signaling cascades; however, the diversity of signaling pathways is not limited to those described earlier. With that said, initiation and the sequence of events in many known cell processes include these signaling pathways. It is worth mentioning that research of most known signaling pathways is generally focused on studying pathological states of organism or it uses cell culture models. There is very little information in the literature about how cell signaling pathways work under normal physiological conditions.

## Pathways for systems biology

### Diverse definitions and concepts of systems biology

Systems biology is a broad term that describes a rapidly developing field of research at the intersection of biology, statistics, mathematics, and computer science. Biology has always been a multidisciplinary science with a systemic approach in which diverse topics merged, thereby helping researchers to better understand processes in individual organisms and their biotic communities. Karl Ludwig von Bertalanffy, Mihajlo D. Mesarović, and the “father” of artificial intelligence theory Norbert Wiener are considered the first ones to combine the ideas of interdisciplinary knowledge into the unified approach of systems biology ([Bertalanffy, 1975](#); [Biggart and Gloveli, 2017](#); [Mesarović, 1968](#); [Wiener, 1961](#)). However, the term itself appeared later and became popular at the junction of millennia ([Boogerd et al., 2007](#); [Zieglgänsberger and Tölle, 1993](#)).

Undoubtedly the emergence of systems biology in its current meaning has to do with the development of biotechnology that allowed the

collection of large volumes of biomolecular data and the advancement of computer technologies for storing and processing previously inconceivable amounts of information. Modern systems biology combines the results of research studies performed in different fields and at different scales including whole genome sequencing projects, the compilation of libraries of metabolic reactions in plants, the analysis of protein expression in individual patients and the reconstruction of molecular ontologies of evolutionary trees.

Systems biology can be defined as the biology of systems, since the system itself is the object of research in this discipline. A system, in its widest definition, is understood as a set of individual elements connected to each other so that those connections define the behavior of the system as a whole. A system is an object in the focus of information theory, cybernetics, synergetics, and general systems theory; all of them develop approaches for the analysis based on the axiom that a system is something more than just a sum of its parts. For instance, the immune system consists of separate cells and proteins with unique properties. However, the specific characteristics of the immune system itself are different from its component elements and can describe the system as a whole. Or, for example, Pyotr Anokhin's theory of functional systems describes connections between organs and processes in the human organism and suggests that we aggregate physiology into a single integrated self-regulated system. This functional systems theory can even be used to explain distinct human behavior (Sudakov, 1997). Also the concept of sustainable development is another good example of when a system displays new features different from the properties of its elements.

Biological systems can be open or closed systems, complex and modular systems, equilibrium systems, or systems with various feedback mechanisms; these are just among of their many characteristics. One of the pioneers in biological systems theory, Alexander Malinovsky, suggested that we distinguish between two extreme categories: "hard systems" where each element is indispensable and "discreet systems" where elements are more or less equivalent. Such a distinction along with identification of intermediate hybrid, star, and net types of systems allowed a researcher to estimate the efficacy and robustness of particular systems by assessing the number of intrinsic links, which alter the behavior of the entire system.

## Pathways as objects for analysis in systems biology

Systems can be represented in different ways, for example, as tree structures, termed ontologies, or as systems of differential equations. Nowadays, systems biology is based on huge amounts of experimentally acquired measurements of molecular data that are most often used for the analysis of systems at the scale of the individual cell and the interactions

between molecules. The general goal of the analysis is to assess and model mechanisms that control interactions between cells, genes, proteins, and chemical reactions and thus define a living organism as an integrated system (more on experiment data analysis in [Chapter 14](#), “Application of Disease Pathways in Biology and Medicine”).

Models are different though. There is still disagreement about the definition of a “model,” however, despite that the modeling approach has been broadly used in biology since 1963 when Hodgkin and Huxley won the Nobel Prize for their model of the generation of the nerve action potential ([Baker et al., 2018](#); [Thakur, 1991](#)).

Models of molecular interactions, among other things, differ in their methods of reconstruction and their purpose of analysis. The purpose of analysis can be described with three paradigms—prediction, classification, and testing—which, in general, help to explain how and what internal factors or external stimuli do influence the studied biological process.

We use the word “prediction” with its universal meaning of discovering novel ideas. Predictive models help to find putative new interactions and new patterns when used to analyze data from biological experiments. Also, models assist the prediction or testing of a hypothesis about the system’s behavior. This type of model can be used for the simulation of real-life events in silico, before experimental verification. In silico simulations support the process of hypothesizing how the biological process would react with distinct stimuli. For example, a dynamic pathway model of biochemical glucose cascades in pancreatic cells may predict this cell type’s response to different doses of treatment. Then, models can be used to classify the studied biological process, and the molecules predicted to be involved can be measured in experiments. For example, modeling techniques that include subdividing networks into clusters, searching overrepresented subnetworks, constructing hierarchical trees, and comparative analysis of models have been explored with the aim of grouping genes and proteins by level of activity measured experimentally to reveal their functional roles in the network ([Kim et al., 2010](#); [Thomas and Bonchev, 2010](#)).

Models of molecular interactions are categorized by the methods used to reconstruct them. A large variety of approaches have been developed for the creation and analysis of biological models. The discussion within traditional mathematical descriptions of the process generalizes any model to be either mechanistic or statistical ([Baker et al., 2018](#); [Hecker et al., 2009](#); [Thakur, 1991](#)). Mechanistic models are mathematical models that are based on a given assumption about the causes of the dynamic process. These models are designed to be deterministic, and they answer the “how” question about the underlying mechanisms of the process (such as chemical reactions, gene cascades, cellular systems, population waves, or pharmacodynamics of the drug). Technically, mechanistic mathematical

models operate a system of differential equations and computational algorithms to describe a predefined order of rules and dynamic changes that occur over time in the modeled process.

Statistical models assume that rules do not control system behavior; rather, they are stochastic and influenced by random events. In practice, describing any biological process needs a hybrid model composed of a combination of deterministic, statistic, quantitative, dynamic, mathematical, and algorithmic attributes. For example, it is important to include statistical estimation in the deterministic “mechanistic” model’s quantitative parameters detected experimentally (Bardini et al., 2017).

One of the most intuitive ways to depict a system takes advantage of the idea of a network that graphically represents elements that are connected by lines. Indeed a network model can be built for any biological system. Graph theory is actively used for the construction, modeling, and analysis of the molecular interactions in biological systems. In graph theory the concepts of “system” and “parts” are equivalent to the concepts of “graph” or of “network” and “nodes,” while the relationships between parts or nodes are called “edges” (Barabási, 2016; Gross et al., 2013). Graph theory-based computer models of cellular systems allow the identification of the most important functional elements in a system, such as key cell types, genes, proteins, metabolic cascades, and principal types of interactions that include the direct binding between key elements and coexpression of multiple elements.

Information theory is used for a similar purpose in the field of systems biology. Network entropy and other concepts from information theory are applied to modeling and analyzing the major cellular communication channels and the amount of information transfer within systems of molecular interactions. This approach addresses the question of what path between stimulus and response, for example, medication and gene expression, is optimal and how it will change depending on the dosage of that medication (Hecker et al., 2009; Mousavian et al., 2016a,b).

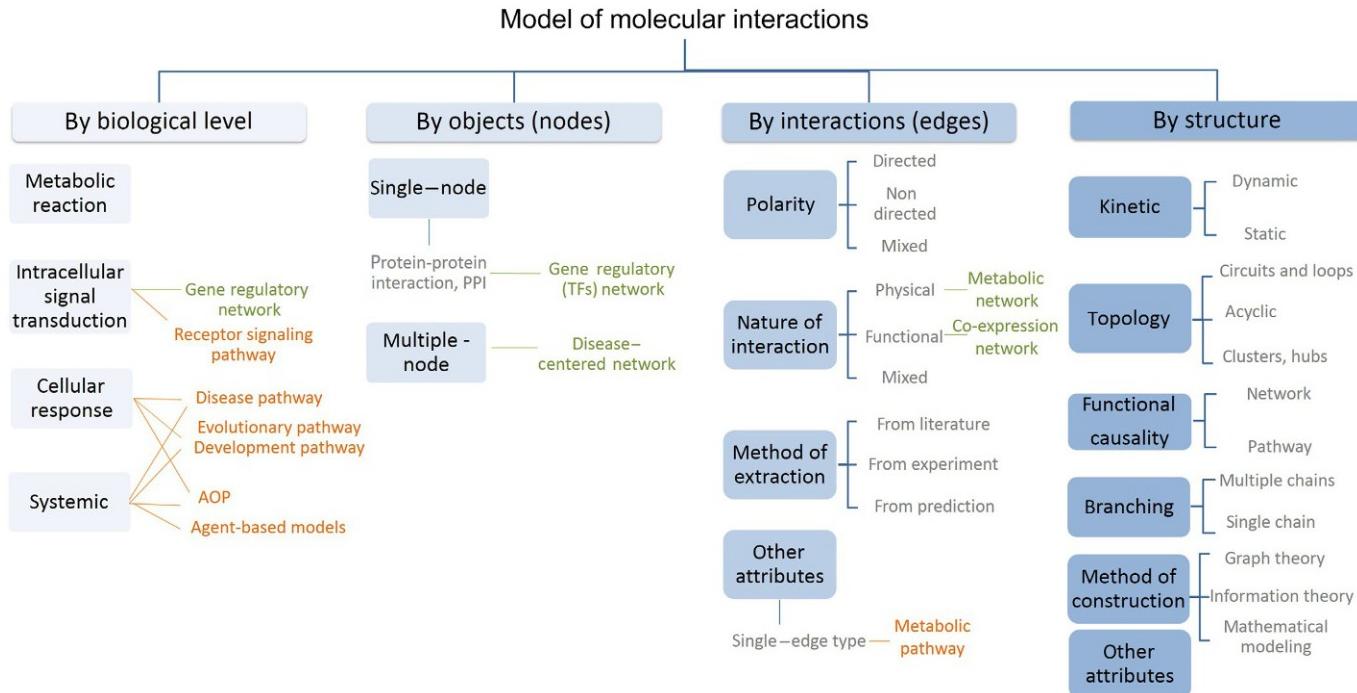
The vector-driven molecular pathway is an appropriate model for this and similar tasks because it has established a topological structure, which can be examined. The vector-driven topology itself is the characteristic that differentiates the pathway model from the network model. However, the “network-pathway” division shares the problem of polysemy with the terms “deterministic mechanistic” and “statistic.” Many properties are shared between pathway and network. The size of the interaction model, the number of nodes and edges, is often used as separating property, but the size criterion is imprecise and does not matter for the definition of a network. Sometimes, it is difficult to separate a subnetwork from the pathway or subpathway. Then the pathway is often defined as a group of proteins working together to perform a particular biological function, and the network is not restricted to specific biological functions. Still, some

“subnetworks” are only used as clusters of genes with specific biological functions. We want to emphasize that the separation depends on the purpose of using the models. Thus, in this book, we use the method of reconstruction as a primary discriminator between the terms “network” and “pathway” since our goal of using pathways is illustrative.

Despite the subdivision of molecular interaction model types into networks and pathways by “causality,” that is, the presence of a vector for the information flow, there are other specifications. Fig. 2 illustrates an attempt to summarize the principles of classifying molecular interaction models by two main principles. The first one is the classification by the level of biological organization. The second one is by elements of the model structure: object (node) centered, interaction (edge) centered, and by topological properties (Fig. 2).

First of all, molecular interaction systems can be categorized according to the level of organization from the lowest levels including genome size and complexity and transcriptome cascades to the highest levels of ecological and evolutionary networks. For example, one can differentiate models that describe biological processes (e.g., renal physiology, lactation, or memory formation) from cellular processes (e.g., apoptosis, histone modification, or cytoskeleton assembly). Graph analyses of complex networks, along with other computational methods, can assess the similarity of system models on different organismal levels that in turn helps to answer questions at the highest level of evolutionary molecular biology. This can help, for example, to identify molecular interactions that are equivalent among different species. Also, models from different species can be used to predict behavior of similar systems in humans, which in turn can be applied to develop new medical remedies.

The classification of molecular models based on the types of objects (nodes) specifies how many types of items can be described by the model. A single-node type model allows only one type of object, for example, a protein, with interactions (edges) between them that represent different molecular relationships (Nguyen et al., 2018). The single-node model with a single type of interaction is used in many methods because it is easy to analyze. An example of such a simple molecular model can be the pathway or network of protein-protein direct binding interactions that were discovered by experimentally using two-hybrid system assays. Although the analysis of real biological experiments needs considerably more information, therefore modern methods try to work with multiple nodes, multiple edges, and multiple properties. Multiple-node models of molecular interactions can describe biological events at different levels of organization. Cell lineage pathways link cells and their developmental precursors with the help of cellular molecular expression profiles. Disease models connect cellular systems and phenotypes through molecular interactions and so on. Boucher and Jenna suggested six different levels of abstraction

**FIG. 2** The classification of pathway molecular models.

in the molecular modeling of genome-phenotype systems. Consistent with that, much effort is being devoted to establish links between networks located at different levels. Boucher and Jenna suggested considering genomic structure as level 1, expression patterns as level 2, physical interactions as level 3, functional interactions as level 4, biological process as level 5, and phenotype as level 6 (Boucher and Jenna, 2013).

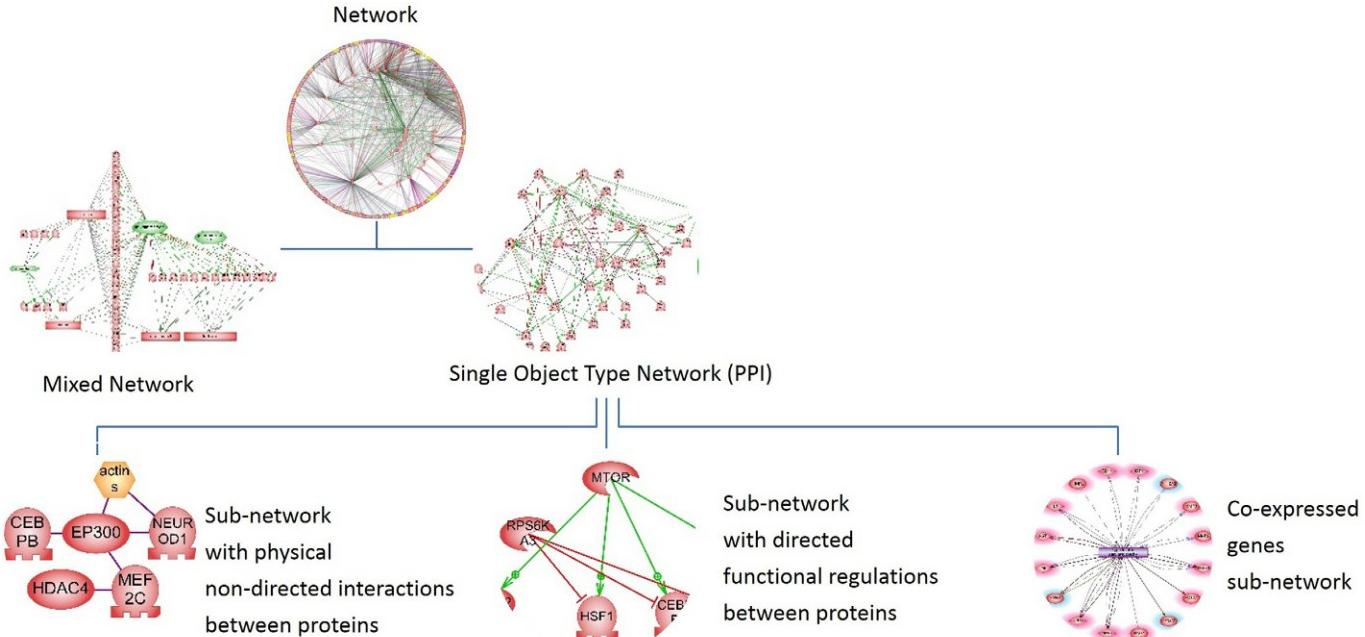
The classification of molecular models based on interactions (edges) takes into account the attributes of relationships between nodes. Edges may be directed or nondirected. Directed ones may possess a stimulatory or inhibiting effect. There are also other properties indicating the strength of the interaction or its reliability, the mechanisms of interaction (e.g., phosphorylation), methods of extraction, or evidence of kinetic attributes. Models can be built using one or several types of edges. Thus metabolic pathways represent the model type that depicts chemical reactions rather than variants of signal transduction.

Most cited types of molecular interaction models include metabolic models, protein-protein interaction (PPI), gene regulatory network (GRN), metabolic and signaling pathways, and coexpression networks (Mieczkowski et al., 2012; Vella et al., 2017). Also, there are dynamic mathematical models of complex systems that may include molecular interactions such as agent-based models or cellular automata (Broderick, 2012; Chen et al., 2014; Chiacchio et al., 2014; Walpole et al., 2013).

Metabolic models include biochemical compounds as nodes. Edges in metabolic models represent reactions that transform the compound(s). Enzymes that carry out the biochemical reactions may also be nodes or may be associated with edges rather than nodes (ElKalaawy and Wassal, 2015). Protein-protein interaction (PPI) is a single-node type model that incorporates different relationships between proteins or genes. Those relationships symbolize physical interactions between molecules or the functional regulatory associations when one molecule influences the function of another, sometimes involving an intermediary (the third one). PPI models are usually not restricted to specific biological functions and often are built in the network form (Fig. 3). Sometimes, models based on functional associations are called “influence” network models (Gardner and Faith, 2005; Hecker et al., 2009).

A gene regulatory network (GRN and transcriptional regulatory network) is a model describing the control of transcription. A gene regulatory network can be considered as an example of PPI with specific interactions between transcription factors and their targets (gene promoters, mRNA, and other proteins). The interactions within such models originate from gene expression patterns that are reconstructed from genome-wide measurement of gene expression or calculated based on protein-DNA binding profiles (Barbosa et al., 2018). GRNs can also be examples of the single edge-centered model type. An example is a model with different types of

## I. Introduction



**FIG. 3** The classification of network molecular models on protein-protein interaction networks (PPI) and mixed networks.

molecules physically bound to gene promoters to initiate the transcription. PPI and GRN networks with physical protein-protein, protein-DNA, or protein-RNA interactions belong to the third level of abstraction, according to the Baucher and Jenna classification. The term “gene regulatory network” often is used in experimental, developmental biology and synthetic microbiology. When applied to humans, due to the challenge of the genome size, the term GRN is more synonymous with a signaling pathway or subnetwork, which highlights gene expression changes (Arda et al., 2013). A human-specific GRN includes both physical and functional interactions describing different levels of gene expression control including direct DNA binding, regulation by RNAs, and coexpression patterns among proteins (e.g., transcription factors and their targets) (Gerstein et al., 2012).

“Coexpression network” models are reconstructed directly from experimental measurements of gene expression from a significant part of the genome. Some authors use GRN and coexpression network synonymously, while others prefer to separate them by physical (GRN) or functional (co-expression) types of interactions (Vella et al., 2017).

Signal transduction models (signaling pathway and subnetwork) focus on the information flow in a system by describing the hypothetical order within the chain of interactions. A signaling pathway can explain metabolic, genetic, protein, cellular, or even higher context levels. A signaling pathway model can be structural and dynamic. Structural (static) network models are based only on the topology of connections (effect, direction, and type), wherein dynamic models also require numerical values (ElKalaawy and Wassal, 2015). Quantitative data such as kinetic rate constants or concentrations of proteins at different time points reveal the steady states and limits of the system, which can prompt the optimal course of further experiments. Kinetic (dynamic) metabolic pathways are typically used in metabolic engineering (Copeland et al., 2012). Models of biological circuits, for example, which include signal transduction pathways with feedback loops in the regulation of gene transcription or cyclic chemical reactions, usually also belong to dynamic model types (Alon, 2006). Agent-based models are the next popular implementation of the dynamic modeling approach. This approach combines mathematical methods (differential equations and statistics) and deterministic, pre-defined rules for object (agents) communications to simulate the collective behavior of agents in time and in response to any stimuli. This approach is used in metabolic engineering, the simulation of dynamic of multiple cellular systems (like the immune system), modeling diseases, and gene evolution. Aged-based modeling and similar techniques are sometimes called synthetic biology because they aim to develop “synthetic systems” in silico that may mimic the regulation of real biological scenarios (Chiachio et al., 2014; Gorochowski, 2016) (more on dynamic modeling in Chapter 13 “Application of disease pathways in biology and medicine”).

Obviously, all computational methods have certain limitations when applied to living things. Within the cell, there are many more interactions between molecules than any single model or hypothetical pathway can cover. Therefore any system with molecular interactions is more complex than its model. Despite the complexity of modern methods, they are often insufficient for the separation of noise in the experimental data from the meaningful patterns or for *in silico* building of comprehensive pathway models (Hecker et al., 2009). Besides, standards for the assembly of biological models and cellular networks have yet to be established. Standards are required for the integration of analytical results produced by different teams. A standard methodology will help to generate models with the least number of unknown molecules and the least uncertainty or ambiguity in the identification of interactions. The use of special markup languages like Systems Biology Markup Language (SBML), Cell Markup Language (CML), or Biological Pathway Exchange (BioPAX) to exchange files of cellular networks and pathways is one of the attempts to achieve this needed standardization. The next step is expected to bring standard tools and software applications for professionals to generate and analyze cellular models. From there, we will establish a unified terminology that will further support efforts to simplify the integration of data from different studies.

## Pathways ontology and disease pathways

Existing network databases and manually created pathway models are used for interpreting experimental molecular data in systems biology and in precision medicine (Petri et al., 2014; Ramanan et al., 2012). The latter is currently one of the main applications of pathway models.

Classification of manually reconstructed pathway models can be done in the form of a tree or ontology, which can be made with different principles in mind. Currently, there are several public resources where pathway ontology is arranged as a set of different categories and pathway types, like Kyoto Encyclopedia of Genes and Genomes (KEGG) or Reactome (Kanehisa, 2000; Vastrik et al., 2007). Those resources differ from each other not only by the way content is classified but also by the models themselves, their number, their properties, by the way they are represented, and the resource's capabilities for their usage. Sometimes a classification of genes on groups that refer to a biological term or another descriptor is considered as pathway ontology. Gene ontology (GO) is the most developed and broadly used such gene-centered vocabulary. It is worth to note that while GO gene sets are useful in data analysis, they do not have relations between genes so cannot be considered as true "pathway ontology" (Ashburner et al., 2000; Gene Ontology Consortium, 2017).

The evolution of approaches to producing molecular pathway models and of ways to analyze them has increased the number of pathway collections. Therefore settling on the criteria for pathway unification and quality assessment becomes an essential task. What is a good pathway model and how should the quality of pathway models could be scored? So far, there is no definitive answer to those questions. If we rely on the philosophical axiom that the goodness can be determined by utility, then the value of the pathway model depends on the primary purposes of the application, and there are multiple applications for pathway collections.

Among the criteria, several key characteristics are most important for generating pathway models. Such as the presence of strictly defined input signals and output effects. Also, unambiguous logical schematics of signal transduction between molecules; or the opposite, a sufficient number of feedback loops. The most important criterion for reconstructed models can also be the use of only physical links between molecules. For example, those are gene promoter activation following transcription factor binding or interactions leading to a change of protein conformation such as phosphorylation. It is also important to avoid indirect links between molecules that imply the presence of an entire cascade of interactions behind the scenes. Avoiding these makes a model less ambiguous and more trustworthy. However, it is not always possible to achieve these goals because of the gaps in scientific knowledge about the cell process or of the need to make a model human readable.

Most of the models in current pathway ontologies are reconstructed manually by specialists in molecular biology; biochemistry; and genetics, using different technical tools. This too makes it hard to standardize and classify the available models. Typical software application for pathway model reconstruction provides different instruments including synchronized with public databases universal identifiers for nodes (i.e., proteins, genes, molecules, and biological processes), the network of molecular interactions for adding established edges, and elements of pathway analysis.

Models of pathological states, such as toxicological or disease pathways, take a special place in any pathway collection or ontology. Firstly, curing diseases is one of the first goals of human biology. Then, without comparing the altered state that we might call unhealthy with the alternative states, we cannot understand what “healthy” pathways are to use them as canonical models.

In general a disease pathway model is built the same way as any other manually reconstructed pathway. The typical method of disease pathway reconstruction generally consists of transferring published information about the model into the software, hence the name “reconstruction.” However, some proteins within disease-related cellular cascades should be either switched off or hyperactivated constantly, thereby altering

normal signal flow. These changes may be annotated in attributes of both the nodes and the edges of the disease pathway model.

A typical disease can rarely be described with just one reconstructed pathway; often, various types of cells, tissues, and organs participate in disease progression, so multiple pathways are required to uncover relevant molecular processes that occur in different parts of an organism. Further, even the most detailed pathway is a simplified model of the real situation; often, it has a specific focus on one or another aspect of the system. This is particularly true for disease pathways. (Although for some well-studied diseases, there might be the opposite problem of the limited abundance of available information to fit within the model.) The level of detail and the depth of the models greatly depend on the state of research for each disease. To reconstruct a reliable model, researchers first aim to identify a consensus within the scientific community; they do not develop theories by themselves. In any case, each of manually reconstructed disease pathways depicts the very key processes and molecular or cellular contributors to the pathway. That would probably be sufficient to achieve a high level of understanding of the disease mechanism, but at the same time, in many cases, the depth and amount of detail in such simplified schemes would be insufficient for genome-level analyses.

Using gene interaction networks for disease modeling can be the answer to this problem. In such case, genes, proteins, or compounds are grouped into disease subnetworks (module, cluster, and hub) based on previous knowledge about their associations with the phenotype and about their coexpression or coregulation patterns. Calculating a disease subnetwork employs the hypothesis that molecules involved in the same disease tend to interact with each other and form dense areas in the network and have a higher degree of node connectivity. A disease model is then seen as a set of subnetworks (modules) of molecules or processes associated with disease (Hao et al., 2018; Oti and Brunner, 2007). The methods of building global molecule–disease networks and subdividing them into specific disease subnetworks are similar to the methods used for building and analyzing interaction networks in general. These approaches include clustering, statistical, graph, and information theory methods (Newman, 2006).

Disease subnetworks (modules), as well as comprehensive manually reconstructed pathways, can be used to classify the disease categories (subtypes) based on their molecular profiles. Disease-related models will also be useful for the prediction of additional useful molecular biomarkers and potential drug targets. Moreover the simulation of dynamic disease models helps to test new therapeutic strategies and formulate hypotheses about the causes of the disease. Dynamic disease subnetworks are best suited to describe disease progression although most often there is insufficient prior knowledge to build dynamic disease models (Hao et al., 2018; Ideker and Sharan, 2008; Kann, 2007; Kontou et al., 2016; Lehner, 2007; Zhou et al., 2018).

The calculation of a network-based disease model requires different knowledge databases, like global molecular interaction networks, manually created pathways, and gene ontologies. These are databases that collect evidence about genotype-phenotype associations within diseases and the genes known to cause them (for example, Online Mendelian Inheritance in Man (OMIM), <https://www.omim.org>, ClinVar, <https://www.ncbi.nlm.nih.gov/clinvar>, and GWAS Catalog, <https://www.ebi.ac.uk/gwas>). The next category of resources includes manually curated ontologies of terms. These are vocabularies with the names of diseases or phenotypes, as well as the genes, which are required for external validation of disease models. Human phenotype ontology (<https://hpo.jax.org/app>), disease ontology (<http://disease-ontology.org>), and medical subject headings (MeSH, <https://meshb-prev.nlm.nih.gov>) are well-accepted examples of structured disease vocabularies. However, other public and commercial projects also can be useful. For example, DISEASES (Pletscher-Frankild et al., 2015), ResNet (Elsevier R&D Solutions, 2018), MalaCards (<https://www.malacards.org>), or DrugBank (<https://www.drugbank.ca>) store disease or drug-centric information extracted from the literature or aggregated from different sources.

## Reconstruction of disease pathways described in the book

The content of this book is based on Elsevier's disease pathways collection. Pathway models reconstructed in Pathway Studio, as opposed to many other large collections, have identifiers and specifications not only for the members of the pathway (nodes) but also for the relationships (edges) among them. Each relationship is supported by at least one citation from original research papers. This way, each pathway model can be considered a literature network for a specific subject. The ability to supplement each relation with a citation and a reference to the actual research is provided by the database that resides at the core of Pathway Studio. This database stores more than 10 million relationships extracted from the biomedical literature using natural language processing technology (NLP). NLP technology identifies subject-verb-object triplets in scientific texts as indicators of meaningful relationships between terms much in the same way humans identify relationships through reading (e.g., ligand-to-receptor relations) (Daraselia et al., 2007). Automated text-mining tools with NLP technology can process millions of full-text scientific articles and abstracts in a matter of hours. Nevertheless, manual curation of a biological thesaurus and of ontologies by domain experts remains a critical factor for achieving high-quality output from automated text processing (more on Pathway Studio data model in "Guide and Legend").

Disease pathways in Pathway Studio are composed of several types that describe pathological changes in cellular mechanisms that occur during a disease, for example, receptor signaling, metabolic cascades, or

subnetworks with mutated genes. For reconstructing the disease pathways provided in this book, the authors took advantage of relations automatically extracted from the literature and stored in Pathway Studio; they also used conventional methods of collecting information that researcher generally use such as summarizing review articles, researching of specialized resources, reading textbooks, and looking for details in the tables presenting experimental data. Thanks to the linking each relationship on the pathway with the database of interactions automatically extracted from the scientific literature, the manually reconstructed Elsevier pathway collection provides a rare example of greater pathway visualization reinforced with updated every 2 weeks citations. Another distinctive feature of Elsevier's pathways is several ontological annotations (labels) that help to find statistically significant associations between patient experimental data and signals specific to cells, organs, tissues, or diseases ([Nesterova and Yuryev, 2017](#)).

A universal method for reconstructing of a pathway for this book includes several typical steps:

Key input and output participants found in the database are added to the model pathway. Those can be diseases, cell processes describing clinical symptoms or pathological changes, known disease triggers (e.g., genes with mutations), or known molecular biomarkers. The database is used to find all the relations between added members along with their neighbors and functions as well as learning the general roles of the chosen objects. Also the database is used for generating groups (lists) with mutated genes associated with the disease, including key molecular players in pathogenesis, and group(s) of drugs and their therapeutic targets.

For example, the general purpose of the pathway "acetaminophen-induced hepatotoxicity" will illustrate the side effect of a widely used medication—namely, the death of liver cells. Acetaminophen (paracetamol) is a widely used antipyretic and analgesic. The small molecule "acetaminophen," the disease "hepatotoxicity," and the cell type "hepatocyte" are principal incoming components of the pathway. Collected information allows for compiling a large list of articles in a matter of minutes. The compiled information helps to guide the workflow. Specifically, how many separate model pathways should be reconstructed and which biological processes should serve as the starting point.

Thus literature analysis along with the database enables researchers to reconstruct the logic and chain of reactions between input and output entities.

For example, acetaminophen is a small molecule that should be metabolized with the help of enzymes, so proteins that have specific chemical relations with acetaminophen should be added to the pathway model. Acetaminophen is metabolized primarily in the liver by cytochrome CYP2E1 into the toxic metabolite acetamidoquinone (*N*-acetyl-*p*-benzoquinone imine, NAPQI). Once the pathway model is initiated, the database can be used to identify proteins that are altered directly by

this molecule and are critical for hepatotoxicity. In hepatocytes, NAPQI is capable of directly modifying cysteine residues within a polypeptide chain by arylation, leading to the inactivation of catalase (CAT) and the inhibition of a  $\text{Ca}^{2+}$ -transporting ATPase (ATP2B1). The depletion of these proteins causes reactive oxygen species to accumulate, which in turn promotes apoptotic mitochondrial damage. These events, together with decreased ATP production, induce necrosis. Another target of NAPQI found in the database is HMG-CoA synthase (HMGCS2), which is one of the key enzymes in ketone biosynthesis. Therefore this metabolic pathway is also blocked by NAPQI.

Often, papers and experiments do not describe the mechanism of cellular signaling completely, so many indirect relations remain in a pathway. In most cases, filling the gaps is possible because for many major cell proteins, the signaling cascades are already well described and can be found in pathway resources (e.g., Elsevier pathway collection has more than 2000 pathways).

The last step in pathway reconstruction is to verify each object in the pathway. This is done by finding additional information in the literature to ensure that proteins are expressed in relevant cell types and that the experiments were conducted using human cells.

## References

- Alcaino, C., Farrugia, G., Beyder, A., 2017. Mechanosensitive piezo channels in the gastrointestinal tract. *Curr. Top. Membr.* 79, 219–244. <https://doi.org/10.1016/bs.ctm.2016.11.003>.
- Alon, U., 2006. An Introduction to Systems Biology: Design Principles of Biological Circuits. CRC Press Book, Chapman & Hall/CRC Mathematical and Computational Biology. Chapman and Hall/CRC.
- Arda, H.E., Benitez, C.M., Kim, S.K., 2013. Gene regulatory networks governing pancreas development. *Dev. Cell* 25, 5–13. <https://doi.org/10.1016/j.devcel.2013.03.016>.
- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., Sherlock, G., 2000. Gene ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29. <https://doi.org/10.1038/75556>.
- Baker, R.E., Peña, J.-M., Jayamohan, J., Jérusalem, A., 2018. Mechanistic models versus machine learning, a fight worth fighting for the biological community? *Biol. Lett.* 14, 20170660. <https://doi.org/10.1098/rsbl.2017.0660>.
- Bal-Price, A., Lein, P.J., Keil, K.P., Sethi, S., Shafer, T., Barenys, M., Fritzsche, E., Sachana, M., Meek, M.E.B., 2017. Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity. *Neurotoxicology* 59, 240–255. <https://doi.org/10.1016/j.neuro.2016.05.010>.
- Barabási, A.-L., 2016. Network Science. Cambridge University Press.
- Barbosa, S., Niebel, B., Wolf, S., Mauch, K., Takors, R., 2018. A guide to gene regulatory network inference for obtaining predictive solutions: underlying assumptions and fundamental biological and data constraints. *Biosystems* 174, 37–48. <https://doi.org/10.1016/j.biosystems.2018.10.008>.
- Barczyk, M., Carracedo, S., Gullberg, D., 2010. Integrins. *Cell Tissue Res.* 339, 269–280. <https://doi.org/10.1007/s00441-009-0834-6>.

- Bardini, R., Politano, G., Benso, A., Di Carlo, S., 2017. Multi-level and hybrid modelling approaches for systems biology. *Comput. Struct. Biotechnol. J.* 15, 396–402. <https://doi.org/10.1016/j.csbj.2017.07.005>.
- Berg, J.M., Tymoczko, J.L., Stryer, L., Stryer, L., 2002. Biochemistry, fifth ed. W.H. Freeman, New York.
- Bertalanffy, L.V., 1975. Perspectives on General System Theory: Scientific-Philosophical Studies. George Braziller.
- Biggart, J., Gloveli, G., 2017. Bogdanov and His Work: A Guide to the Published and Unpublished Works of Alexander A Bogdanov (Malinovsky) 1873–1928. Taylor & Francis.
- Boogerd, F., Bruggeman, F.J., Hofmeyr, J.-H.S., Westerhoff, H.V., 2007. Systems Biology: Philosophical Foundations. Elsevier.
- Boucher, B., Jenna, S., 2013. Genetic interaction networks: better understand to better predict. *Front. Genet.* 4. <https://doi.org/10.3389/fgene.2013.00290>.
- Bridgham, J.T., Eick, G.N., Larroux, C., Deshpande, K., Harms, M.J., Gauthier, M.E.A., Ortlund, E.A., Degnan, B.M., Thornton, J.W., 2010. Protein evolution by molecular tinkering: diversification of the nuclear receptor superfamily from a ligand-dependent ancestor. *PLoS Biol.* 8. <https://doi.org/10.1371/journal.pbio.1000497>.
- Broderick, G., 2012. A moving target: taking aim at the regulatory dynamics of illness. *Brain Behav. Immun.* 26, 1045–1046. <https://doi.org/10.1016/j.bbri.2012.06.013>.
- Calebiro, D., Godbole, A., 2018. Internalization of G-protein-coupled receptors: implication in receptor function, physiology and diseases. *Best Pract. Res. Clin. Endocrinol. Metab.* 32, 83–91. <https://doi.org/10.1016/j.beem.2018.01.004>.
- Catterall, W.A., 2010. Ion channel voltage sensors: structure, function, and pathophysiology. *Neuron* 67, 915–928. <https://doi.org/10.1016/j.neuron.2010.08.021>.
- Chen, X., Yang, J., Evans, P.M., Liu, C., 2008. Wnt signaling: the good and the bad. *Acta Biochim. Biophys. Sin.* 40, 577–594.
- Chen, X., Hickling, T.P., Vicini, P., 2014. A mechanistic, multiscale mathematical model of immunogenicity for therapeutic proteins: Part 1—Theoretical model. *CPT Pharmacometrics Syst. Pharmacol.* 3, e133. <https://doi.org/10.1038/psp.2014.30>.
- Cheng, Y.-R., Jiang, B.-Y., Chen, C.-C., 2018. Acid-sensing ion channels: dual function proteins for chemo-sensing and mechano-sensing. *J. Biomed. Sci.* 25, 46. <https://doi.org/10.1186/s12929-018-0448-y>.
- Chiacchio, F., Pennisi, M., Russo, G., Motta, S., Pappalardo, F., 2014. Agent-based modeling of the immune system: NetLogo, a promising framework. *Biomed. Res. Int.* 2014. <https://doi.org/10.1155/2014/907171>.
- Copeland, W.B., Bartley, B.A., Chandran, D., Galdzicki, M., Kim, K.H., Sleight, S.C., Maranas, C.D., Sauro, H.M., 2012. Computational tools for metabolic engineering. *Metab. Eng.* 14, 270–280. <https://doi.org/10.1016/j.ymben.2012.03.001>.
- Crino, P.B., 2015. mTOR signaling in epilepsy: insights from malformations of cortical development. *Cold Spring Harb. Perspect. Med.* 5, 4. <https://doi.org/10.1101/cshperspect.a022442>.
- Daraselia, N., Yuryev, A., Egorov, S., Mazo, I., Ispolatov, I., 2007. Automatic extraction of gene ontology annotation and its correlation with clusters in protein networks. *BMC Bioinform.* 8, 243. <https://doi.org/10.1186/1471-2105-8-243>.
- Duc, N.M., Kim, H.R., Chung, K.Y., 2015. Structural mechanism of G protein activation by G protein-coupled receptor. *Eur. J. Pharmacol.* 763, 214–222. <https://doi.org/10.1016/j.ejphar.2015.05.016>.
- ElKalaawy, N., Wassal, A., 2015. Methodologies for the modeling and simulation of biochemical networks, illustrated for signal transduction pathways: a primer. *Biosystems* 129, 1–18. <https://doi.org/10.1016/j.biosystems.2015.01.008>.
- Elsevier R&D Solutions, 2018. Case study: mining text to deliver answers on demand. In: Elsevier Text Mining. <https://www.elsevier.com/solutions/professional-services/text-mining>.

- Favarolo, M.B., López, S.L., 2018. Notch signaling in the division of germ layers in bilaterian embryos. *Mech. Dev.* <https://doi.org/10.1016/j.mod.2018.06.005>.
- Gardner, T.S., Faith, J.J., 2005. Reverse-engineering transcription control networks. *Phys Life Rev* 2, 65–88. <https://doi.org/10.1016/j.plrev.2005.01.001>.
- Gene Ontology Consortium, 2017. Expansion of the gene ontology knowledgebase and resources. *Nucleic Acids Res.* 4, D331–D338. <https://doi.org/10.1093/nar/gkw1108>.
- Gerstein, M.B., Kundaje, A., Hariharan, M., Landt, S.G., Yan, K.-K., Cheng, C., Mu, X.J., Khurana, E., Rozowsky, J., Alexander, R., Min, R., Alves, P., Abyzov, A., Addleman, N., Bhardwaj, N., Boyle, A.P., Cayting, P., Charos, A., Chen, D.Z., Cheng, Y., Clarke, D., Eastman, C., Euskirchen, G., Fretze, S., Fu, Y., Gertz, J., Grubert, F., Harmanci, A., Jain, P., Kasowski, M., Lacroute, P., Leng, J.J., Lian, J., Monahan, H., O'Geen, H., Ouyang, Z., Partridge, E.C., Patacsil, D., Pauli, F., Raha, D., Ramirez, L., Reddy, T.E., Reed, B., Shi, M., Slifer, T., Wang, J., Wu, L., Yang, X., Yip, K.Y., Zilberman-Schapira, G., Batzoglou, S., Sidow, A., Farnham, P.J., Myers, R.M., Weissman, S.M., Snyder, M., 2012. Architecture of the human regulatory network derived from ENCODE data. *Nature* 489, 91–100. <https://doi.org/10.1038/nature11245>.
- Gilmore, T.D., 2006. Introduction to NF- $\kappa$ B: players, pathways, perspectives. *Oncogene* 25, 6680–6684. <https://doi.org/10.1038/sj.onc.1209954>.
- Goldfinger, L.E., 2008. Choose your own path: specificity in Ras GTPase signaling. *Mol. BioSyst.* 4, 293–299. <https://doi.org/10.1039/b716887>.
- Gorochowski, T.E., 2016. Agent-based modelling in synthetic biology. *Essays Biochem.* 60, 325–336. <https://doi.org/10.1042/EBC20160037>.
- Gough, N.R., 2010. Focus issue: endocrine signaling from clinic to cell. *Sci. Signal.* 3, eg9. <https://doi.org/10.1126/scisignal.3143eg9>.
- Gross, J.L., Yellen, J., Zhang, P., 2013. *Handbook of Graph Theory, Discrete Mathematics and Its Applications*, second ed. Chapman and Hall/CRC.
- Handly, L.N., Pilko, A., Wollman, R., 2015. Paracrine communication maximizes cellular response fidelity in wound signaling. *eLife* 4, e09652. <https://doi.org/10.7554/eLife.09652>.
- Hao, T., Wang, Q., Zhao, L., Wu, D., Wang, E., Sun, J., 2018. Analyzing of molecular networks for human diseases and drug discovery. *Curr. Top. Med. Chem.* 18, 1007–1014. <https://doi.org/10.2174/1568026618666180813143408>.
- Hecker, M., Lambeck, S., Toepfer, S., van Someren, E., Guthke, R., 2009. Gene regulatory network inference: data integration in dynamic models—a review. *Biosystems* 96, 86–103. <https://doi.org/10.1016/j.biosystems.2008.12.004>.
- Heine, M., Ciuraszkiewicz, A., Voigt, A., Heck, J., Bikbaev, A., 2016. Surface dynamics of voltage-gated ion channels. *Channels* 10, 267–281. <https://doi.org/10.1080/19336950.2016.1153210>.
- Hoffmann, A., Natoli, G., Ghosh, G., 2006. Transcriptional regulation via the NF- $\kappa$ B signaling module. *Oncogene* 25, 6706–6716. <https://doi.org/10.1038/sj.onc.1209933>.
- Ideker, T., Sharan, R., 2008. Protein networks in disease. *Genome Res.* 18, 644–652. <https://doi.org/10.1101/gr.071852.107>.
- Jacob, L., Lum, L., 2007. Hedgehog signaling pathway. *Sci. STKE* 2007, cm6. <https://doi.org/10.1126/stke.4072007cm6>.
- Kanehisa, M., 2000. *Post-Genome Informatics*. Oxford University Press, Oxford; New York.
- Kann, M.G., 2007. Protein interactions and disease: computational approaches to uncover the etiology of diseases. *Brief. Bioinform.* 8, 333–346. <https://doi.org/10.1093/bib/bbm031>.
- Kim, E.K., Choi, E.-J., 2010. Pathological roles of MAPK signaling pathways in human diseases. *Biochim. Biophys. Acta* 1802, 396–405. <https://doi.org/10.1016/j.bbadi.2009.12.009>.
- Kim, T.Y., Kim, H.U., Lee, S.Y., 2010. Data integration and analysis of biological networks. *Curr. Opin. Biotechnol.* 21, 78–84. <https://doi.org/10.1016/j.copbio.2010.01.003>.
- Kiu, H., Nicholson, S.E., 2012. Biology and significance of the JAK/STAT signalling pathways. *Growth Factors* 30, 88–106. <https://doi.org/10.3109/08977194.2012.660936>.

- Komiya, Y., Habas, R., 2008. Wnt signal transduction pathways. *Organogenesis* 4, 68–75.
- Kontou, P.I., Pavlopoulou, A., Dimou, N.L., Pavlopoulos, G.A., Bagos, P.G., 2016. Network analysis of genes and their association with diseases. *Gene* 590, 68–78. <https://doi.org/10.1016/j.gene.2016.05.044>.
- Lefkowitz, R.J., 2013. A brief history of G-protein coupled receptors (Nobel Lecture). *Angew. Chem. Int. Ed. Engl.* 52, 6366–6378. <https://doi.org/10.1002/anie.201301924>.
- Lehner, B., 2007. Modelling genotype–phenotype relationships and human disease with genetic interaction networks. *J. Exp. Biol.* 210, 1559–1566. <https://doi.org/10.1242/jeb.002311>.
- Lemmon, M.A., Schlessinger, J., 2010. Cell signaling by receptor tyrosine kinases. *Cell* 141, 1117–1134. <https://doi.org/10.1016/j.cell.2010.06.011>.
- Mesarović, M.D., 1968. Systems theory and biology—view of a theoretician. In: *Systems Theory and Biology*. Springer, Berlin, Heidelberg, pp. 59–87. [https://doi.org/10.1007/978-3-642-88343-9\\_3](https://doi.org/10.1007/978-3-642-88343-9_3).
- Mieczkowski, J., Swiatek-Machado, K., Kaminska, B., 2012. Identification of pathway deregulation–gene expression based analysis of consistent signal transduction. *PLoS One* 7, e41541. <https://doi.org/10.1371/journal.pone.0041541>.
- Moreira, I.S., 2014. Structural features of the G-protein/GPCR interactions. *Biochim. Biophys. Acta* 1840, 16–33. <https://doi.org/10.1016/j.bbagen.2013.08.027>.
- Mousavian, Z., Díaz, J., Masoudi-Nejad, A., 2016a. Information theory in systems biology. Part II: Protein-protein interaction and signaling networks. *Semin. Cell Dev. Biol.* 51, 14–23. <https://doi.org/10.1016/j.semcdb.2015.12.006>.
- Mousavian, Z., Kavousi, K., Masoudi-Nejad, A., 2016b. Information theory in systems biology. Part I: Gene regulatory and metabolic networks. *Semin. Cell Dev. Biol.* 51, 3–13. <https://doi.org/10.1016/j.semcdb.2015.12.007>.
- Nesterova, A., Yuryev, A., 2017. Androgenic alopecia: cross-talk between cell signal transduction pathways. In: *Hair and Scalp Disorders*. IntechOpen, pp. 141–174.
- Newman, M.E.J., 2006. Modularity and community structure in networks. *Proc. Natl. Acad. Sci. U. S. A.* 103, 8577–8582. <https://doi.org/10.1073/pnas.0601602103>.
- Nguyen, T., Mitrea, C., Draghici, S., 2018. Network-based approaches for pathway level analysis. *Curr. Protoc. Bioinform.* 61, 8.25.1–8.25.24. <https://doi.org/10.1002/cpbi.42>.
- Nicolas, C.S., Amici, M., Bortolotto, Z.A., Doherty, A., Csaba, Z., Fafouri, A., Dournaud, P., Gressens, P., Collingridge, G.L., Peineau, S., 2013. The role of JAK-STAT signaling within the CNS. *JAK-STAT* 2, e22925. <https://doi.org/10.4161/jkst.22925>.
- Oti, M., Brunner, H.G., 2007. The modular nature of genetic diseases. *Clin. Genet.* 71, 1–11. <https://doi.org/10.1111/j.1399-0004.2006.00708.x>.
- Pearson, G., Robinson, F., Beers Gibson, T., Xu, B.E., Karandikar, M., Berman, K., Cobb, M.H., 2001. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr. Rev.* 22, 153–183. <https://doi.org/10.1210/edrv.22.2.0428>.
- Petri, V., Jayaraman, P., Tutaj, M., Hayman, G.T., Smith, J.R., De Pons, J., Laulederkind, S.J., Lowry, T.F., Nigam, R., Wang, S.-J., Shimoyama, M., Dwinell, M.R., Munzenmaier, D.H., Worthey, E.A., Jacob, H.J., 2014. The pathway ontology—updates and applications. *J. Biomed. Semant.* 5, 7. <https://doi.org/10.1186/2041-1480-5-7>.
- Pletscher-Frankild, S., Pallejà, A., Tsafou, K., Binder, J.X., Jensen, L.J., 2015. DISEASES: text mining and data integration of disease–gene associations. *Methods* 74, 83–89. <https://doi.org/10.1016/j.ymeth.2014.11.020>.
- Polychronidou, E., Vlachakis, D., Vlamos, P., Baumann, M., Kossida, S., 2015. Notch signaling and ageing. *Adv. Exp. Med. Biol.* 822, 25–36. [https://doi.org/10.1007/978-3-319-08927-0\\_6](https://doi.org/10.1007/978-3-319-08927-0_6).
- Rajagopal, S., Rajagopal, K., Lefkowitz, R.J., 2010. Teaching old receptors new tricks: biasing seven-transmembrane receptors. *Nat. Rev. Drug Discov.* 9, 373–386. <https://doi.org/10.1038/nrd3024>.
- Ramanan, V.K., Shen, L., Moore, J.H., Saykin, A.J., 2012. Pathway analysis of genomic data: concepts, methods, and prospects for future development. *Trends Genet.* 28, 323–332. <https://doi.org/10.1016/j.tig.2012.03.004>.

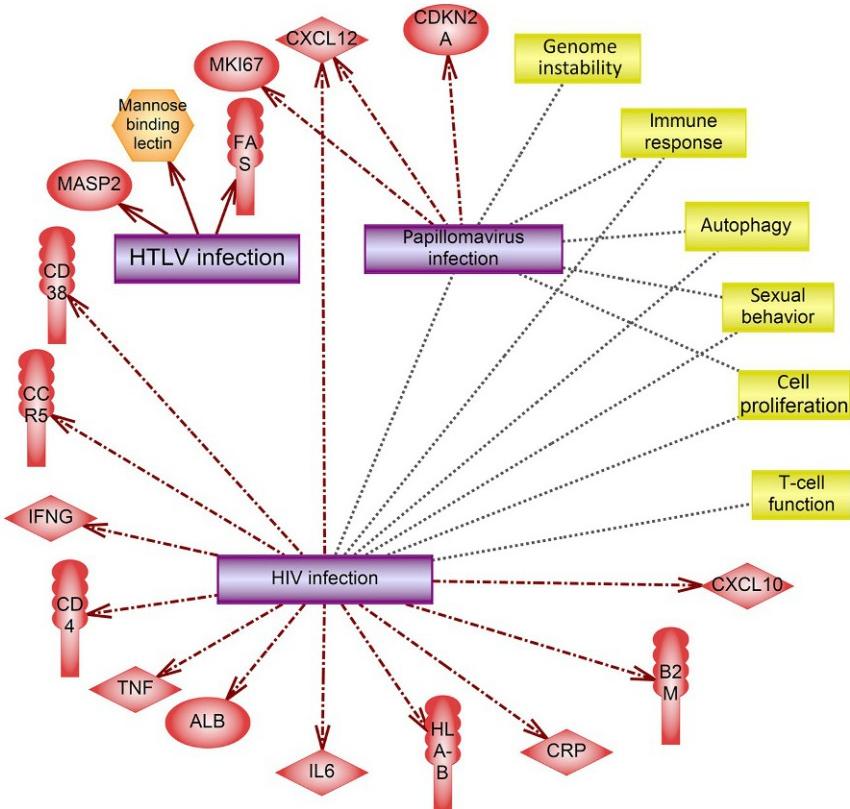
- Re, R.N., 2003. The intracrine hypothesis and intracellular peptide hormone action. *BioEssays News Rev. Mol. Cell. Dev. Biol.* 25, 401–409. <https://doi.org/10.1002/bies.10248>.
- Rocks, O., Peyker, A., Bastiaens, P.I.H., 2006. Spatio-temporal segregation of Ras signals: one ship, three anchors, many harbors. *Curr. Opin. Cell Biol.* 18, 351–357. <https://doi.org/10.1016/j.ceb.2006.06.007>.
- Schomburg, D., Michal, G. (Eds.), 2012. *Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology*. second ed.. John Wiley & Sons, Hoboken, NJ.
- Sudakov, K.V., 1997. The theory of functional systems: general postulates and principles of dynamic organization. *Integr. Physiol. Behav. Sci.* 32, 392–414. <https://doi.org/10.1007/BF02688634>.
- Thakur, A.K., 1991. Model: mechanistic vs empirical. In: Rescigno, A., Thakur, A.K. (Eds.), *New Trends in Pharmacokinetics*. NATO ASI Series, Springer US, Boston, MA, pp. 41–51. [https://doi.org/10.1007/978-1-4684-8053-5\\_3](https://doi.org/10.1007/978-1-4684-8053-5_3).
- Thomas, S., Bonchev, D., 2010. A survey of current software for network analysis in molecular biology. *Hum. Genomics* 4, 353. <https://doi.org/10.1186/1479-7364-4-5-353>.
- Vastrik, I., D'Eustachio, P., Schmidt, E., Joshi-Tope, G., Gopinath, G., Croft, D., de Bono, B., Gillespie, M., Jassal, B., Lewis, S., Matthews, L., Wu, G., Birney, E., Stein, L., 2007. Reactome: a knowledge base of biologic pathways and processes. *Genome Biol.* 8, R39. <https://doi.org/10.1186/gb-2007-8-3-r39>.
- Vazquez, J.P., Pulido, E.G., Aparicio, L.M.A., 2012. Cytokine and endocrine signaling in prostate cancer. *Med. Oncol.* 29, 1956–1963. <https://doi.org/10.1007/s12032-011-0036-4>.
- Vella, D., Zoppis, I., Mauri, G., Mauri, P., Di Silvestre, D., 2017. From protein-protein interactions to protein co-expression networks: a new perspective to evaluate large-scale proteomic data. *EURASIP J. Bioinforma. Syst. Biol.* 2017, 6. <https://doi.org/10.1186/s13637-017-0059-z>.
- Volinsky, N., Kholodenko, B.N., 2013. Complexity of receptor tyrosine kinase signal processing. *Cold Spring Harb. Perspect. Biol.* 5, a009043. <https://doi.org/10.1101/cshperspect.a009043>.
- Walpole, J., Papin, J.A., Peirce, S.M., 2013. Multiscale computational models of complex biological systems. *Annu. Rev. Biomed. Eng.* 15, 137–154. <https://doi.org/10.1146/annurev-bioeng-071811-150104>.
- Webb, S.D., Owen, M.R., 2004. Intra-membrane ligand diffusion and cell shape modulate juxtacline patterning. *J. Theor. Biol.* 230, 99–117. <https://doi.org/10.1016/j.jtbi.2004.04.024>.
- Wiener, N., 1961. *Cybernetics or Control and Communication in the Animal and the Machine*. MIT Press.
- Yu, C., Woo, H.J., Yu, X., Oyama, T., Wallqvist, A., Reifman, J., 2017. A strategy for evaluating pathway analysis methods. *BMC Bioinform.* 18, 453. <https://doi.org/10.1186/s12859-017-1866-7>.
- Zeng, H., Chi, H., 2014. mTOR signaling and transcriptional regulation in T lymphocytes. *Transcription* 5, e28263. <https://doi.org/10.4161/trns.28263>.
- Zhou, X., Lei, L., Liu, J., Halu, A., Zhang, Y., Li, B., Guo, Z., Liu, G., Sun, C., Loscalzo, J., Sharma, A., Wang, Z., 2018. A systems approach to refine disease taxonomy by integrating phenotypic and molecular networks. *EBioMedicine* 31, 79–91. <https://doi.org/10.1016/j.ebiom.2018.04.002>.
- Zieglgänsberger, W., Tölle, T.R., 1993. The pharmacology of pain signalling. *Curr. Opin. Neurobiol.* 3, 611–618.

## P A R T II

# Human disease pathways

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# Infectious diseases



## O U T L I N E

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In general, infectious diseases can be described as diseases that can be spread from one person to another and are caused by bacteria, viruses, fungi, and other parasitic organisms (World Health Organization (WHO), [https://www.who.int/topics/infectious\\_diseases](https://www.who.int/topics/infectious_diseases)). The treatment of viral infections is a well-known challenge, and therefore their pathogenesis has been intensively investigated. In this chapter, we review three examples of relatively well-understood viral infections.

Viruses are intracellular parasites, which rely on proteins and other cellular building blocks of the host cell to replicate themselves. The interactions of viral proteins and the viral genome with human polymerases, transcription factors, and other proteins that regulate cell cycle progression is the main focus of research on the pathogenesis of viral infections.

There are several key steps during the development of any viral infection. At first the virus must penetrate the host cell; then the viral genomes often integrate into the host genome and, finally, activation of viral gene transcription and replication. The suppression of cellular antiviral response and of the host's immune response to viral proteins is important as well. Viruses must support the survival of an infected cell for successful replication; therefore resistance to apoptosis of infected cells is considered another critical research topic.

The listed mechanisms have been studied in detail for many viruses. Human immunodeficiency virus (HIV) is the most well-studied one due to the social concerns about the related acquired immunodeficiency syndrome (AIDS). T cells are the primary target cell type for HIV. Human T-cell leukemia virus (HTLV) is similar to HIV because it is also an RNA-containing virus that affects T cells. Comparing HIV and HTLV infections gives insights into different strategies employed by similar viruses.

Human papillomavirus (HPV) is responsible for the most common viral infection of the reproductive tract according to WHO. Two types of HPV (types 16 and 18) cause most cases of cervical cancer and are responsible for precancerous cervical lesions. From a biological point of view, HPV provides an example of the pathogenesis of a DNA-containing virus that targets nonimmune human cells.

## CHAPTER

## 2.1

## Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS)

Acquired immunodeficiency syndrome (AIDS) is a result of infection by the human immunodeficiency virus (HIV), which progressively weakens the cellular immune system leading to the development of secondary (opportunistic) infections and/or malignancies.

The human immunodeficiency virus (HIV) is a retrovirus that is responsible for causing acquired immunodeficiency syndrome (AIDS). HIV infection does not necessarily mean a person has AIDS. (*Ferri and Ferri, 2018*).

HIV is a single-stranded RNA lentivirus (a genus of retroviruses) categorized as either type 1 or 2. While HIV-1 is the predominant pathogenic retrovirus in humans, disease caused by HIV-2 tends to progress less rapidly than that of HIV-1. HIVs are transmitted by sexual contact, contact with blood or other body fluids of infected individuals, from mother to child during pregnancy, baby delivery, or breastfeeding.

Symptoms of HIV infection or AIDS depend on the stage of the disease. During the earliest acute stage of HIV infection (which occurs during the first weeks following infection until the formation of HIV-specific antibodies), signs of viral infection may manifest due to high levels of systemic viral replication and a permanent loss of mucosa-associated CD4+ T cells, which express the glycoprotein cluster of differentiation 4 (CD4) and C-C chemokine receptor type 5 (CCR5).

During the chronic stage, which lasts 8–10 years, the levels of viral replication decrease. Chronic HIV infection is usually asymptomatic but may include nonspecific symptoms such as lymphadenopathy, fatigue, weight loss, diarrhea, and different skin changes such as dermatitis or signs of opportunistic viral and fungal infections (*Mogensen et al., 2010; Sugden et al., 2016*). Advanced disease is characterized by other opportunistic infections, indicator diseases, and malignancies such as pneumonia and lymphoma (*Sokoya et al., 2017*).

If the HIV infection is confirmed, AIDS is diagnosed with a CD4+ T-cell count <14% of total lymphocytes. Although, based on the Department

of Health and Human Services (DHHS) Guidelines of 2015, active antiretroviral therapy (ART) of HIV should be started with any CD4 cell count. During combined ART the level of plasma HIV falls below the level of detection, which is 50 copies of viral RNA per 1 mL of plasma (Van Lint et al., 2013). ART helps prevent AIDS development and turns it from a life-threatening to a chronic disease (Ferri and Ferri, 2018).

Genetics can change the HIV sensitivity and disease progression. Individuals with deletions in the CCR5 gene, which encodes a cell surface receptor needed for HIV entry into a host cell, have delayed disease progression (Li et al., 2014). Also, one infected person in 300 individuals can maintain a normal CD4+ T-cell count. That individual is referred to as an “elite controller.” Elite controllers are a heterogeneous group of people with strong anti-HIV immune responses due to polymorphisms in the HLA genes and related effective CD8+ T-cell response (Sokoya et al., 2017; Van Lint et al., 2013).

HIV targets several cell types including T-cell subsets, monocytes, macrophages, and dendritic cells (DCs) through different recognition mechanisms. However, the primary target of HIV is mucosal CD4+ memory T cells expressing CD4+ and CCR5 and mucosal macrophages expressing CCR5. The formation of viral synapses and the accumulation of HIV in mucosal macrophages are important for HIV progression. Following HIV penetration of the host cell, the virus modulates transcriptional regulation, mRNA processing, intracellular protein transport, and the cytoskeleton to maintain viral reproduction.

**Pathway 1. HIV entrance to the host cell and viral reproduction (Fig. 1).** In the acute stage of HIV infection, the rapid replication of HIV in its target cells leads to the irreversible depletion of short-lived mucosal CD4+ memory T cells that have a half-life of less than a day. Then the virus reproduces in the next population of cells with a half-life of 1–4 weeks. The acute stage of HIV infection lasts no more than several weeks before the peak of viremia declines. Infected cells die by apoptosis induced through a combination of stress imposed by viral replication and immune system activation. HIVs are also able to delay apoptosis and the elimination of infected cells by CD8+ T cells to prolong the release of viral particles.

**Pathway 2. HIV-induced depletion of CD4+ T cells (Fig. 2).** Resting memory CD4+ T cells and other cells with extended half-lives and cells resistant to viral-induced apoptosis maintain stores of provirus particles and host the production of virions episodically (blips) during the chronic phase of viral infection (Van Lint et al., 2013).

**Pathway 3. HIV latency and evasion of reservoir cell apoptosis in HIV chronic phase.** Low level of viral production (Fig. 3).

Postintegration latency (Fig. 4).

## Key cellular contributors and processes

### Apoptosis

#### Process

Apoptosis is a highly regulated chain of events leading to cell destruction that occurs in multicellular organisms. Apoptosis eliminates damaged or redundant cells and is required for normal tissue development and homeostasis.

### Effector memory T cells

#### Cell

Effector memory T cells are a subset of antigen-experienced T cells that can be distinguished from another subset of memory T cells, the central memory T cells, by the presence of different protein markers expressed on their surface. Effector memory T cells can enter sites of inflammation outside of the lymphoid tissue.

### HIV latency

#### Process

HIV latency is characterized by a transcriptionally silent viral genome, which retains its ability to reactivate and replicate.

### Tissue-resident T cells

#### Cell

Tissue-resident T cells are a subset of T cells that reside in tissues without recirculating. They provide enhanced protection against infections that enter through body surfaces.

### Viral replication

#### Process

Viral replication is the production of new viruses inside infected host cells.

### Basal epithelial cell

#### Cell

Basal cells are found in the deepest (basal) layer epithelia.

### Caveolae

#### Anatomic structure

Caveolae are small invaginations found in the plasma membrane of a variety of cell types and are involved in signal transduction and membrane trafficking.

## Pathway 1

### HIV entrance to the host cell and viral reproduction (Fig. 1)

#### Inducing signals

After being transferred to the human body, HIV first penetrates the mucosa resident memory T cells expressing both CD4 and CCR5 and the macrophages that are recruited to the infected area.

Viral envelope glycoproteins (env gp120 and env gp141) bind to CD4+ and CCR5 receptors on the host cell membrane, thereby triggering membrane fusion and HIV entry into the cell. The formation of viral synapses between T cells can be another way of HIV-1 transmission. Penetration into cells through clathrin-mediated endocytosis is also possible for HIV-1. Dendritic cells may transfer HIV-1 to T cells using DC-SIGN signaling to capture the virus ([Dutartre et al., 2016](#)).

The HIV-1 envelope encloses a cone-shaped capsid with two copies of the positive ssRNA and several copies of viral proteins including reverse transcriptase (RT) and integrase, as well as two cellular tRNAs. All viral molecules are injected into the cell following membrane fusion ([Mogensen et al., 2010](#)).

The HIV-1 retroviral genome carries the *env* gene, which encodes glycoproteins of the envelope (codes gp160 protein); the *gag* gene, which encodes structural group-specific antigen (gag) proteins of the matrix and capsid; and the *pol* gene, which encodes the enzymes polymerase, integrase, and transcriptase. Then, viral *tat* and *rev* genes are responsible for DNA transcription and RNA transport. Lastly, accessory genes such as *vif*, *vpr*, *nef*, *vpu*, and *vpx* are necessary for virus replication in some host cells. HIV-1 has the *vpu* gene while HIV-2—the *vpr* gene. Overall the HIV-1 mRNA encodes 15 proteins ([Ferguson et al., 2002](#)).

#### Outcome effects

The life cycle of HIV-1 can be divided into two phases: the early phase occurs between the time of entry into the host cell and integration into its genome, while the late phase occurs from the time of provirus integration of integrated provirus to that of full viral replication. The provirus integrated into the genome either reproduces with the help of the host proteins or stays in the latent form.

An infected T cell may transfer HIV-1 to another T cell by viral synapses. Viral synapses are complex formations that are established following the engagement of HIV-1 envelope glycoproteins on the membrane of infected cells with their receptors on nearby target cells. Both virally

expressed and host proteins are needed for the formation of viral synapses (the forming of viral synapses is not shown on the pathway) (Dutartre et al., 2016; Gropelli et al., 2015).

Cells expressing CLEC4M (DC-SIGN) are the first cells infected by HIV. These cells are dendritic cells, monocytes, and macrophages, which load virions in the infectious state and in turn transmit them to T cells (Landi et al., 2011).

## Signaling

### **HIV-1 entry**

The HIV gp160 protein is cleaved into gp120 and gp41 by the human protease FURIN during the viral replication cycle. To form an active fusion protein, gp120 and gp41 polypeptides remain noncovalently bound together. This interaction is often not stable, leading to shedding of soluble gp120 and membrane-bound gp41 proteins. Env gp120 binds to the CD4+ receptor on the T-cell membrane surface. There is evidence that some of HIVs use the CXCR4 receptor for cell entry (Friedrich et al., 2011). Several other proteins like filamin A (FLNA) were also reported to be important for binding of HIV to the cell membrane (Ospina Stella and Turville, 2018).

Upon the attachment and fusion of the viral envelope with the cell membrane, the capsid enters the cytoplasm where it uncoats and releases viral RNAs and proteins. Several cellular proteins including cyclophilin A (PPIA) and peptidylprolyl cis/trans isomerase (PIN1) can recognize capsid peptides and support the process of viral uncoating, although the exact mechanism of this activity is not well known (Friedrich et al., 2011).

### **Transport to the nucleus and viral integration**

Inside the cell the preintegration complex (PIC), consisting of both viral and cellular proteins, is transported into the nucleus. Viral reverse transcriptase (RT), the Gag matrix protein (p17), Vpr, and integrase are accompanied by two copies of viral RNA. During the translocation of PIC, RT synthesizes a linear double-stranded DNA copy of the single-stranded viral genome in a complex process known as reverse transcription.

During the translocation and after the completion of reverse transcription, the cellular proteins barrier to autointegration factor 1 (BAF), HDGFRP2, and PSIP1 (LEDGF) recruit and orientate the PIC onto the chromatin. In the nucleus, viral cDNA integrates into chromosomal DNA with the help of the viral integrase. Integrated viral cDNA, named the provirus, is required for its replication. If the HIV-1

provirus does not integrate into the host chromosome, it circularizes, and the virus will not reproduce in that cell. The processes of cDNA integration and genome replication by integrase and RT produce errors or mutations and are the reasons for HIV-1 genetic variability. Also, recombination between viral DNA molecules when infection involves different virus strains results in the release of HIV subtypes (Ferguson et al., 2002).

### **Viral reproduction**

The transcription of integrated virus genomes and translation of virally encoded mRNAs starts with the help of the cellular machinery. So, HIV production will start only upon activation of host transcription factors. The process also requires host cell-derived translation proteins and ribosomes for the synthesis of viral proteins. NF- $\kappa$ B, SP1, and NFAT1 are recruited to viral sequences to promote transcription of viral genes. As HIV is dependent on the host cell for its own replication and expression of its own genes, actively proliferating T cells are important for viral production (Friedrich et al., 2011). Several human proteins are known to inhibit HIV-1 replication, for example, the apolipoprotein B mRNA-editing enzyme catalytic subunit 3G (APOBEC3G) (Desimmie et al., 2014).

The viral rev (regulator of expression of virion proteins) protein is expressed shortly after infection as a fully spliced mRNA. Incompletely spliced viral mRNAs do not efficiently exit the nucleus. Rev moves intron-containing viral mRNAs from the nucleus to the cytoplasm via the exportin 1 (XPO1) nuclear export pathway (Holmes et al., 2015).

The transactivator of transcription (tat) protein is also expressed shortly after infection. The tat protein binds replicated viral mRNA transcripts and interacts with T-cell-expressed proteins (see Pathway 2).

The *nef* gene localizes in the plasma and nuclear membranes of infected cells and activates host NF- $\kappa$ B, thereby triggering viral gene expression.

The same mRNA encodes both the *pol* and *gag* genes, which are translated into different proteins. The *pol* gene encodes proteins with enzymatic functions. The *gag* protein is posttranslationally cleaved into several peptides including the capsid protein matrix (MA, p17), nucleocapsid (NC, p7), capsid (CA, p24), and core proteins p1, p2, and p6. *Vif*, *vpr*, *nef*, *vpu*, and *vpx* are also expressed (not shown on the pathway). Finally, viral components eventually assemble into new infectious particles.

## II. Human disease pathways

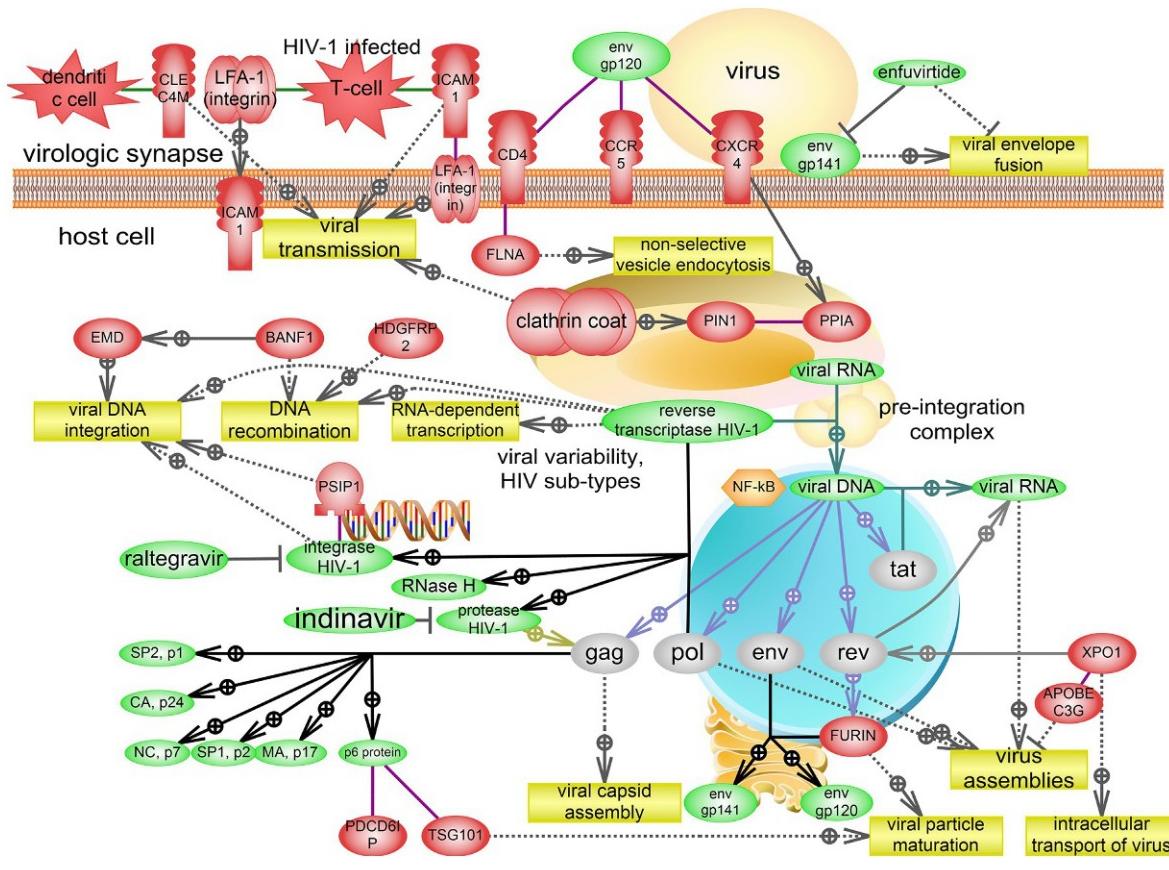


FIG. 1 Pathway 1: HIV entrance to the host cell and viral reproduction.

## Pathway 2

### HIV-induced depletion of CD4+ T cells (Fig. 2)

#### Inducing signals

HIV involves an adaptive strategy to control host gene expression during its reproduction. A number of host proteins participate in viral replication and reproduction. Depending on the stage of the virus life cycle, viral proteins may act as either inducers or inhibitors of cell death ([Timilsina and Gaur, 2016](#)). During the early stages, viral proteins prevent apoptosis to maintain viral replication, whereas during the late stages, viral proteins activate apoptosis to facilitate virus release. The timeframes for the early and late stages are not long. From 6 to 48 hours following viral entry are needed for reverse transcription of the viral genome, and 5 more hours are needed for the viral genome integration. The duration of the postintegration phases of the viral cycle and the average lifetime of infected cells is assumed to be about 48 hours ([Holmes et al., 2015](#)).

HIV infection triggers the depletion of CD4+ T cells by different mechanisms including a direct attack of viral proteins on the T cells, abnormal T-cell trafficking, clonal exhaustion of T cells, drainage of memory T-cell pools, and chronic immune activation. Yet the major mechanism for HIV driven CD4+ T-cell depletion is programmed cell death (apoptosis). Indirectly, HIV infection activates immune system cells (CD8+ T cell, NK cell, B cell, and dendritic cell) through the production of inflammatory cytokines and viral proteins by infected T cells. CD8+ T cells (cytotoxic T lymphocytes) play the most prominent role in suppressing HIV infection ([Vidya Vijayan et al., 2017](#)).

The HIV-1 envelope proteins glycoprotein gp120 (gp120), transactivator of transcription (Tat), negative regulatory factor (Nef), viral protein R (Vpr), and viral protein unique (Vpu) are effective in modulating host cell signaling pathways to control viral particle production.

#### Outcome effects

In the acute stage of HIV infection, the selective and dramatic depletion of CD4+ CCR5+ effector memory T cells occurs predominantly from mucosal surfaces. In the chronic stage of infection, the elimination of T cells continues. Acute HIV infection does not efficiently target naïve and resting central memory T-cell populations, leaving the potential open for the regeneration of new CD4+ CCR5 T cells. In the gut, naïve and central memory T cells are short lived and have limited regenerative potential, so they can only partially substitute for CD4+ effector memory T cells. As a result the weak immunological barrier in the mucosa of the gastrointestinal tract

allows microbial products to get in the blood leading to chronic infection and immune activation.

## Signaling

HIVs need to tightly regulate transcription and translation to allow efficient viral gene expression and inhibit host cell antiviral gene expression.

NF-κB is an essential transcription factor in the HIV reproduction cycle. NF-κB binds to viral LTR promoters where it initiates efficient viral gene expression. Most subtypes of HIV-1 contain two NF-κB binding sites. Transcribed viral RNA and translated viral proteins are sensed by intracellular signaling pathways, which then activate NF-κB.

Not only activated NF-κB regulates the expression of specific proteins, but also it stimulates apoptosis in T cells. Substantial evidence was collected on viral proteins, which activate or inhibit T-cell apoptosis and NF-κB signaling. Only a few mechanisms are shown on this pathway.

The proteins Tat and Nef expressed during early stages of the viral reproductive cycle have been shown to facilitate the activation of NF-κB signaling directly. It has been reported that Tat promotes expression of the p65 subunit of NF-κB, which binds DNA. The modulation of NF-κB signaling with both stimulatory and inhibitory effects by Env, Vpr, and other viral proteins has been described ([Heusinger and Kirchhoff, 2017](#)).

Tat is the key HIV protein implicated in CD4+ T-cell apoptosis ([Février et al., 2011](#)). Tat association with the PTEN and PP2A promoters is considered the initiating event of Tat-mediated apoptosis ([Dabrowska et al., 2008; Février et al., 2011](#)).

The activation of PTEN-FOXO3A by Tat reactivates the membrane-bound tumor necrosis factor receptor superfamily (TNFRSF10) and the Fas cell surface death receptor (FAS) and, therefore, their apoptotic signaling pathways ([Timilsina and Gaur, 2016](#)). The acetylation of Tat by host acetyltransferase CREB-binding protein (CREBBP) and CBP-associated factor (PCAF) enhances its activity ([Coiras et al., 2009](#)). The apoptosis of T cells is mediated mostly by caspase-3 (CASP3). Tat activates caspase-8 and increases intracellular BAX activity.

The viral Env protein activates CCR5 signaling through binding to the receptor on the cell surface. CCR5 transduces its signal through PI3K and modulates intracellular calcium levels leading to the activation of transcription factors and reorganization of the cytoskeleton. CCR5 promotes the synthesis of proapoptotic tumor necrosis factors and FASLG in T cells and macrophages. Macrophage-released FASLG binds to FAS on T cells causing them to undergo apoptosis.

The virally encoded Nef also guides the balance between the activating and inhibitory pathways in T cells to permit optimal viral replication. On the one hand, Nef activates T cell binding to the serine/threonine kinase

p21-activated kinase 2 (PAK2) and MAP3K5 stimulating proliferative signals. On the other hand, Nef binds to the membrane trafficking adaptor proteins (clathrin adaptor AP-1) and thus reorganizes the structure or facilitates degradation of cell surface receptors. For example, Nef alters the subcellular localization of major histocompatibility complex type I (MHC-I) molecules and CD28, and it protects T cells from cytotoxic CD8+ T cells. CD8+ T cells are responsible for the removal of virus-infected cells from the body by inducing apoptosis in HIV-infected CD4+ cells ([Landi et al., 2011; Pawlak et al., 2018](#)). Since stimulation of the T-cell receptor and the CD28 receptor is also important for the PKC-dependent activation of NF- $\kappa$ B and other transcriptional factors, Nef indirectly inhibits HIV replication to avoid superinfection (not shown for simplicity).

The viral protein Vpr, which is expressed in late stages of the viral reproduction cycle, arrests infected cells at the G2 phase of the cell cycle and induces apoptosis by binding to BAX. Fusion of the viral Env protein (gp120) with CD4+ T-cell surface receptors leads to increased expression of levels of TNF, TNFRSFs, and also Fas, resulting in the activation of apoptosis ([Cummins and Badley, 2010; Timilsina and Gaur, 2016](#)).

The viral protein U (Vpu) is a membrane protein expressed later than Nef and Tat in the viral reproduction cycle. Vpu has a function similar to Nef that results in the removal of MHC-1 and other cell surface receptors from the T-cell surface. In addition, Vpu hijacks bone marrow stromal antigen 2 (BST2) trafficking. BST2 is involved in the intracellular antiviral response (see [Pathway 3](#)).

At the same time, Vpu can suppress NF- $\kappa$ B activity during the later stages of viral replication cycle. Vpu stabilizes IkB and reduces translocation of the p65 subunit of NF- $\kappa$ B to the nucleus. Vpu effects are dominant over the effects of Nef and Tat, which activate NF- $\kappa$ B in the early stages of viral replication ([Heusinger and Kirchhoff, 2017](#)).

## II. Human disease pathways

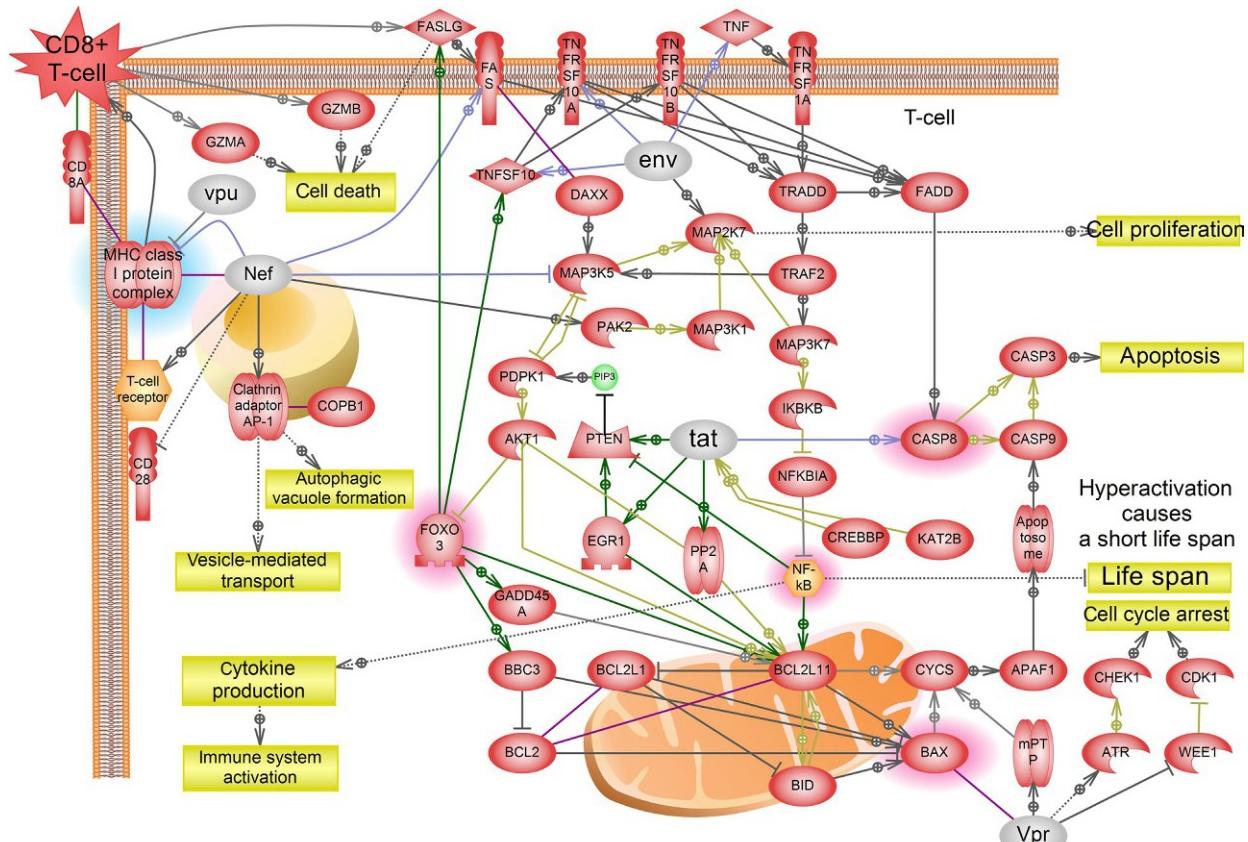


FIG. 2 Pathway 2: HIV-induced depletion of CD4+ T-cells.

## Pathway 3

### HIV latency

#### Inducing signals

The ability of HIV to use cells as viral reservoirs by keeping the infection in the latent form is the biggest challenge in the treatment of HIV infection. Although viral proteins facilitate apoptosis in mucosal CD4+ T cells, other host cells can avoid apoptosis or avoid recognition by the immune system, thus surviving to serve as reservoirs for HIV. Infected macrophages, resting central memory T cells, transitional memory T cells, follicular dendritic cells, hematopoietic progenitor cells, and even astrocytes and microglia can act as HIV reservoirs (Nikitina et al., 2018; Timilsina and Gaur, 2016).

The postintegration latency during which provirus integrates into the cell's genome but is not transcribed has been given more attention since this state may persist steadily for several years. In the majority of latently infected cells, HIV-1 infection appears to be blocked at this postintegration transcriptional level. Latency at the posttranscriptional level due to the inhibition of nuclear RNA export and the inhibition of HIV-1 translation by microRNAs is also possible (Coiras et al., 2009; Mbonye and Karn, 2014).

However, in HIV reservoirs such as long-lived macrophages, viruses are not completely silent, but instead, they maintain a low level of replication or a low level of production of viral particles.

HIV-1 infects macrophages by interacting with CD4, CXCR4, CCR5, or C-type 1 (hMRC1) and DC-SIGN receptors and also by endocytosis (Machado Andrade and Stevenson, 2018; Nikitina et al., 2018)

#### Outcome effects

Latent HIV-1 proviruses are insensitive to antiviral therapy and to the host immune response. Infected cells with silent viruses become a permanent source for virus reactivation. Mechanisms of HIV latency and of stable reproduction are still under investigation.

#### Signaling

##### **Low level of viral production (Fig. 3)**

Monocytes and macrophages are not suitable viral reservoirs since they are nondividing cells and have a short half-life. However, monocytes are the first cells that contact the virus, and after that, they become capable of differentiating into stable, long-lived macrophages, which transfer HIV to other tissues. HIV infection changes the macrophage's intracellular pathways to block their apoptosis and prolong their life span. However,

the mechanism of HIV-1 latency in monocytes is not fully understood (Fernández Larrosa et al., 2008; Mbonye and Karn, 2014).

The expression of intracellular full-length Tat (Tat101) instead of Tat encoded by only the first exon (Tat72) may prompt protection against apoptosis. The Tat101-mediated antiapoptotic effects may occur through stabilization of the mitochondrial membrane and the linked impaired caspase-3 activation. Also, Tat101 probably upregulates antiapoptotic gene expression through the activation of NF- $\kappa$ B and PI3K signaling. The antiapoptotic proteins X-linked inhibitor of apoptosis protein (XIAP), BCL2, baculoviral IAP repeat-containing 3 (BIRC3), and CFLAR (c-FLIP) can play an important role in the survival of latently infected cells as compared with uninfected cells (Berro et al., 2007; López-Huertas et al., 2013).

CFLAR binds to and inhibits FADD and/or caspase-8/caspase-10. Also, CFLAR probably defeats HIV by modulating the expression of viral restriction factors such as the apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G), the bone marrow stromal cell antigen 2 (BST2), and the tripartite motif protein 5 alpha (TRIM5A). CFLAR may directly block trafficking of the viral envelope protein gp120 and the viral capsid protein Gag p24 into lipid rafts (Strebel et al., 2009; Tan et al., 2013).

Perhaps, viral replication is not the reason for the resistance to apoptosis observed in monocytes and macrophages. The modulation of mitochondrial BAX-related pathways and the low susceptibility to apoptotic stimuli, such as oxidative stress in general, can be an alternative explanation (Fernández Larrosa et al., 2008).

In addition, HIV infection shifts macrophage differentiation from the M1 type to the M2 type, which results in high levels of IL-4, TGFA, and IL-10 (Nikitina et al., 2018).

### **Postintegration latency (Fig. 4)**

Resting central memory T cells (defined as CD45RA– CCR7+ CD27+) and translational memory T cells (defined as CD45RA– CCR7– CD27+) are thought to be the prominent reservoirs of latent proviruses in HIV infection.

Establishing postintegration viral latency depends on the process of the integration of viral DNA into the host genome. HIV-1 selectively integrates into intronic regions of actively transcribed genes. To achieve this, LEDGF/p75 interacts directly with the virally encoded integrase.

Several mechanisms leading to transcriptional interference during the integration process (called promoter occlusion, enhancer trapping, or steric hindrance) may lead to the following transcriptional repression and therefore latency of HIV (Colin and Van Lint, 2009).

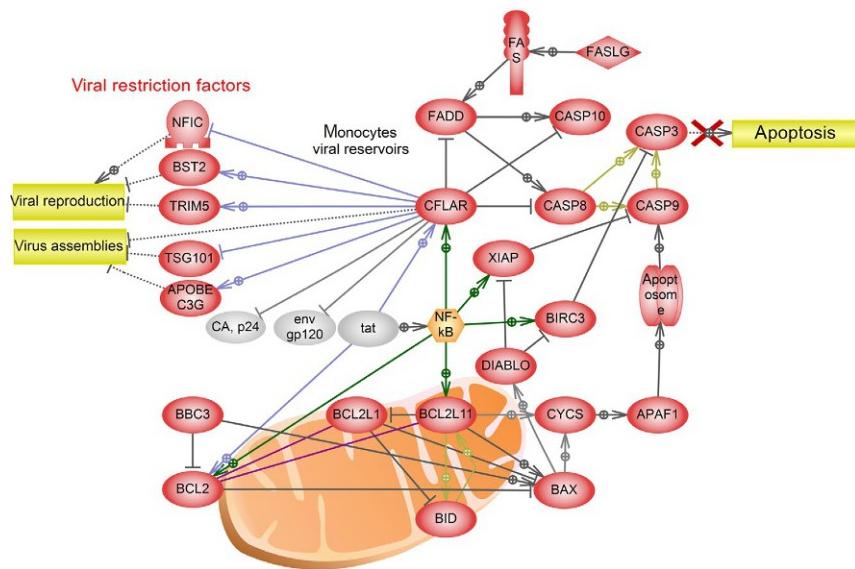
The initiation of HIV transcription requires several cellular transcription factors, including those that are ubiquitously expressed, such as Sp1 and TFIID, and some that are induced by extracellular signals, such as

NF-κB. Therefore, in nonactivated quiescent T cells, the lack of available cellular transcription factors may contribute to viral latency.

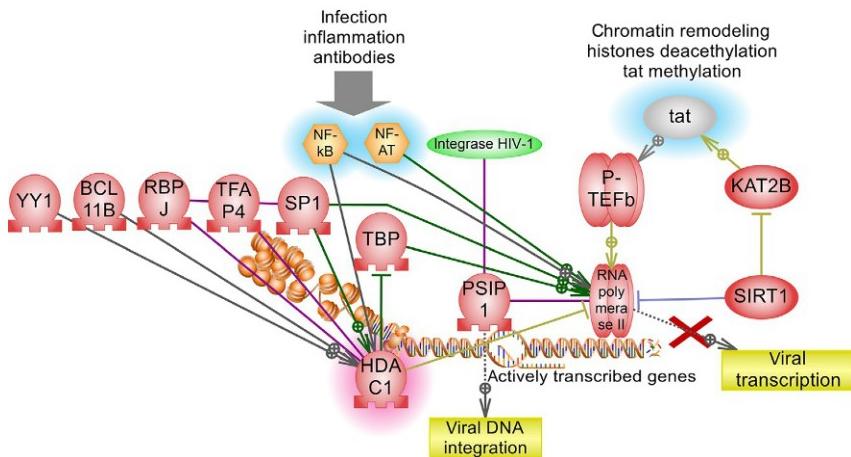
Secondly, there are mechanisms that change chromatin organization of the HIV-1 promoter region into a repressive state. The deacetylation of histones in nucleosomes nuc-0 and nuc-1 is an important step in the chromatin remodeling needed to suppress the site of HIV integration. Among other nuclear factors, NF-κB p50/p50 homodimers, Ying Yang 1 (YY1), and transcription factor activating protein 4 (TFAP4) may recruit histone deacetylase 1 (HDAC1) to repress HIV-1 transcription in latently infected cells.

Finally, inefficient transcription and elongation of full-length HIV transcripts, due to insufficient levels of the viral protein Tat, can contribute to HIV latency. In the absence of Tat, HIV transcription is initiated but blocked at the early elongation stage. For successful viral transcription, Tat must bind to positive transcription elongation factor b (pTEFb), composed of cyclin T1 and cyclin-dependent kinase 9 (CDK9). Lysine acetyltransferase 2B (KAT2B or PCAF) enhances this recruitment. CDK9 phosphorylates the RNA polymerase II, promoting efficient elongation. At the end of the elongation process, a class III protein deacetylase sirtuin 1 (SIRT1) deacetylates Tat and allows its dissociation from the RNA polymerase II and PCAF complex for a new cycle of transcriptional activation ([Kumar et al., 2015](#)).

Additional factors influencing HIV latency include epigenetic silencing (methylation) of HIV promoters and effects of short interfering RNAs and microRNAs (not shown on the figure), which add one more level of regulation of viral replication ([Coiras et al., 2009](#)).



**FIG. 3** Pathway 3: HIV latency and evasion of reservoir cell apoptosis in HIV chronic phase. Low level of viral production.



**FIG. 4** Pathway 3: HIV latency and evasion of reservoir cell apoptosis in HIV chronic phase. Postintegration latency.

## References

- Disease number #609423 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- Berro, R., de la Fuente, C., Klase, Z., Kehn, K., Parvin, L., Pumfrey, A., Agbottah, E., Vertes, A., Nekhai, S., Kashanchi, F., 2007. Identifying the membrane proteome of HIV-1 latently infected cells. *J. Biol. Chem.* 282, 8207–8218. <https://doi.org/10.1074/jbc.M606324200>.
- Coiras, M., López-Huertas, M.R., Pérez-Olmeda, M., Alcamí, J., 2009. Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs. *Nat. Rev. Microbiol.* 7, 798–812. <https://doi.org/10.1038/nrmicro2223>.
- Colin, L., Van Lint, C., 2009. Molecular control of HIV-1 postintegration latency: implications for the development of new therapeutic strategies. *Retrovirology* 6, 111. <https://doi.org/10.1186/1742-4690-6-111>.
- Cummins, N.W., Badley, A.D., 2010. Mechanisms of HIV-associated lymphocyte apoptosis: 2010. *Cell Death Dis.* 1, e99. <https://doi.org/10.1038/cddis.2010.77>.
- Dabrowska, A., Kim, N., Aldovini, A., 2008. Tat-induced FOXO3a is a key mediator of apoptosis in HIV-1-infected human CD4+ T lymphocytes. *J. Immunol.* 181, 8460–8477.
- Desimmie, B.A., Delviks-Frankenberry, K.A., Burdick, R.C., Qi, D., Izumi, T., Pathak, V.K., 2014. Multiple APOBEC3 restriction factors for HIV-1 and one Vif to rule them all. *J. Mol. Biol., Antiviral Innate Immunity (Part II)* 426, 1220–1245. <https://doi.org/10.1016/j.jmb.2013.10.033>.
- Dutartre, H., Clavière, M., Journo, C., Mahieux, R., 2016. Cell-free versus cell-to-cell infection by human immunodeficiency virus type 1 and human T-lymphotropic virus type 1: exploring the link among viral source, viral trafficking, and viral replication. *J. Virol.* 90, 7607–7617. <https://doi.org/10.1128/JVI.00407-16>.
- Ferguson, M.R., Rojo, D.R., von Lindern, J.J., O'Brien, W.A., 2002. HIV-1 replication cycle. *Clin. Lab. Med.* 22, 611–635.
- Fernández Larrosa, P.N., Croci, D.O., Riva, D.A., Bibini, M., Luzzi, R., Saracco, M., Mersich, S.E., Rabinovich, G.A., Peralta, L.M., 2008. Apoptosis resistance in HIV-1 persistently-infected cells is independent of active viral replication and involves modulation of the apoptotic mitochondrial pathway. *Retrovirology* 5, 19. <https://doi.org/10.1186/1742-4690-5-19>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1 <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Février, M., Dorgham, K., Rebollo, A., 2011. CD4+ T cell depletion in human immunodeficiency virus (HIV) infection: role of apoptosis. *Viruses* 3, 586–612. <https://doi.org/10.3390/v3050586>.
- Friedrich, B.M., Dziuba, N., Li, G., Endsley, M.A., Murray, J.L., Ferguson, M.R., 2011. Host factors mediating HIV-1 replication. *Virus Res.* 161, 101–114. <https://doi.org/10.1016/j.virusres.2011.08.001>.
- Groppelli, E., Starling, S., Jolly, C., 2015. Contact-induced mitochondrial polarization supports HIV-1 virological synapse formation. *J. Virol.* 89, 14–24. <https://doi.org/10.1128/JVI.02425-14>.
- Heusinger, E., Kirchhoff, F., 2017. Primate lentiviruses modulate NF-κB activity by multiple mechanisms to fine-tune viral and cellular gene expression. *Front. Microbiol.* 8. <https://doi.org/10.3389/fmicb.2017.00198>.
- Holmes, M., Zhang, F., Bieniasz, P.D., 2015. Single-cell and single-cycle analysis of HIV-1 replication. *PLoS Pathog.* 11, e1004961. <https://doi.org/10.1371/journal.ppat.1004961>.
- Kumar, A., Darcis, G., Van Lint, C., Herbein, G., 2015. Epigenetic control of HIV-1 post integration latency: implications for therapy. *Clin. Epigenetics* 7, 103. <https://doi.org/10.1186/s13148-015-0137-6>.
- Landi, A., Iannucci, V., Nuffel, A.V., Meuwissen, P., Verhasselt, B., 2011. One protein to rule them all: modulation of cell surface receptors and molecules by HIV Nef. *Curr. HIV Res.* <http://www.eurekaselect.com/94869/article>. (Accessed June 11, 2018).

- Li, H., Liu, T.-J., Hong, Z.-H., 2014. Gene polymorphisms in CCR5, CCR2, SDF1 and RANTES among Chinese Han population with HIV-1 infection. *Infect. Genet. Evol.* 24, 99–104. <https://doi.org/10.1016/j.meegid.2014.03.009>.
- López-Huertas, M.R., Mateos, E., Sánchez del Cojo, M., Gómez-Esquer, F., Díaz-Gil, G., Rodríguez-Mora, S., López, J.A., Calvo, E., López-Campos, G., Alcamí, J., Coiras, M., 2013. The presence of HIV-1 tat protein second exon delays Fas protein-mediated apoptosis in CD4+ T lymphocytes. *J. Biol. Chem.* 288, 7626–7644. <https://doi.org/10.1074/jbc.M112.408294>.
- Machado Andrade, V., Stevenson, M., 2018. Host and viral factors influencing interplay between the macrophage and HIV-1. *J. NeuroImmune Pharmacol.* <https://doi.org/10.1007/s11481-018-9795-4>.
- Mbonye, U., Karn, J., 2014. Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. *Virology* 454–455, 328–339. <https://doi.org/10.1016/j.virol.2014.02.008>.
- Mogensen, T.H., Melchjorsen, J., Larsen, C.S., Paludan, S.R., 2010. Innate immune recognition and activation during HIV infection. *Retrovirology* 7, 54. <https://doi.org/10.1186/1742-4690-7-54>.
- Nikitina, E., Larionova, I., Choinzonov, E., Kzhyshkowska, J., 2018. Monocytes and macrophages as viral targets and reservoirs. *Int. J. Mol. Sci.* 19. <https://doi.org/10.3390/ijms19092821>.
- Ospina Stella, A., Turville, S., 2018. All-round manipulation of the actin cytoskeleton by HIV. *Viruses* 10. <https://doi.org/10.3390/v10020063>.
- Pawlak, E.N., Dirk, B.S., Jacob, R.A., Johnson, A.L., Dikeakos, J.D., 2018. The HIV-1 accessory proteins Nef and Vpu downregulate total and cell surface CD28 in CD4+ T cells. *Retrovirology* 15, 6. <https://doi.org/10.1186/s12977-018-0388-3>.
- Sokoya, T., Steel, H.C., Nieuwoudt, M., Rossouw, T.M., 2017. HIV as a cause of immune activation and immunosenescence. *Mediat. Inflamm.* 2017. <https://doi.org/10.1155/2017/6825493>.
- Strebel, K., Luban, J., Jeang, K.-T., 2009. Human cellular restriction factors that target HIV-1 replication. *BMC Med.* 7, 48. <https://doi.org/10.1186/1741-7015-7-48>.
- Sugden, S.M., Bego, M.G., Pham, T.N.Q., Cohen, É.A., 2016. Remodeling of the host cell plasma membrane by HIV-1 Nef and Vpu: a strategy to ensure viral fitness and persistence. *Viruses* 8, 67. <https://doi.org/10.3390/v8030067>.
- Tan, J., Wang, X., Devadas, K., Zhao, J., Zhang, P., Hewlett, I., 2013. Some mechanisms of FLIP expression in inhibition of HIV-1 replication in Jurkat cells, CD4+ T cells and PBMCs. *J. Cell. Physiol.* 228, 2305–2313. <https://doi.org/10.1002/jcp.24397>.
- Timilsina, U., Gaur, R., 2016. Modulation of apoptosis and viral latency—an axis to be well understood for successful cure of human immunodeficiency virus. *J. Gen. Virol.* 97, 813–824. <https://doi.org/10.1099/jgv.0.000402>.
- Van Lint, C., Bouchat, S., Marcello, A., 2013. HIV-1 transcription and latency: an update. *Retrovirology* 10, 67. <https://doi.org/10.1186/1742-4690-10-67>.
- Vidya Vijayan, K.K., Karthigeyan, K.P., Tripathi, S.P., Hanna, L.E., 2017. Pathophysiology of CD4+ T-cell depletion in HIV-1 and HIV-2 infections. *Front. Immunol.* 8. <https://doi.org/10.3389/fimmu.2017.00580>.

## CHAPTER

## 2.2

## Human T-cell leukemia virus infection and leukemia progression

Human T-cell leukemia virus type 1 (HTLV-1) was the first human retrovirus described. HTLV-1 was identified in 1980 as the causative agent of cutaneous T-cell lymphoma.

HTLV is an RNA virus with a diploid genome composed of two copies of single-stranded RNA. In the host cell the viral RNA is copied into a single-stranded DNA, and subsequently a double-stranded DNA integrates into the host genome to function as a provirus.

There exist four types of HTLV viruses with HTLV-1 being the most common. HTLV-1 infects various types of cells; however, it is most effective infecting CD4+ cells (Matsuoka and Jeang, 2011).

The HTLVs are transmitted through sexual contact, blood transfusions, the use of nonsterile injection needles, and mother's milk. The HTLV-1 and HTLV-2 viruses infect 15–20 million people worldwide, yet there are still no vaccines for the HTLVs (Gonçalves et al., 2010).

HTLV-1 may cause blood cancer, specifically T-cell leukemia (adult T-cell leukemia/lymphoma (ATLL)) and HTLV-1-associated myelopathy (HAM). HAM is an inflammation of the spinal nerves that leads to a loss of coordination and weakness in the legs. Dermatitis, myelopathy, uveitis (eye inflammation), disseminated strongyloidiasis, alveolitis, arthritis, and several other diseases have also been linked to HTLV-1 infection. The HTLV-1 virus is most common in central Africa, South America, and other regions throughout the world (<http://www.hntl1.eu>).

HTLV-2 is associated with some neurological disorders and with chronic pulmonary infections, but there are no invariably associated diseases for HTLV-3 and HTLV-4.

The HTLV-1 genome, flanked by long terminal repeats (LTR) at its 5' and 3' ends, contains several open reading frames (ORF). The *gag*, *pol*, and *env* genes encode structural proteins of the virus. The *pol* gene encodes a protein with reverse transcriptase, protease, and integrase activities, *gag* encodes structural virion proteins, and *env* encodes the SU and TM proteins of the viral envelope.

The pX region of the viral genome, located between *env* and the 3'LTR, contains a group of ORFs encoding viral regulatory factors. These virus proteins—tax, rex, p12, p13, p30, and p21—interact with proteins of infected CD4+ T cells and are responsible for the oncogenic activity of the virus.

The HTLVs can enter the cell in the form of an intact virion. The viral envelope proteins interact with cellular membrane proteins leading to viral entry. Alternatively, viral genomic RNA can be transmitted from one cell to another through a virological synapse.

**Pathway 1.** *HTLV-1 enters the cell* ([Fig. 5](#)).

Interaction of viral and cellular proteins is needed for transcription and replication of the viral genome.

**Pathway 2.** *Transcription and reproduction of the HTLV-1 genome* ([Fig. 6](#)).

Expression of the major viral protein Tax disrupts the cell cycle, impairs DNA repair, and causes aneuploidy.

**Pathway 3.** *Tax-induced aneuploidy in T cells infected with HTLV-1* ([Fig. 7](#)).

**Pathway 4.** *Tax-induced disruption of the G1/S transition in HTLV-1 infected T cell* ([Fig. 8](#)).

Also, Tax is a potent oncogenic protein that modifies the NF- $\kappa$ B signaling pathway, blocks apoptosis, and promotes cell survival.

**Pathway 5.** *Tax-mediated dysregulation of NF- $\kappa$ B signaling in HTLV-1 infection* ([Fig. 9](#)).

The viral protein p12I stimulates degradation of MHC-I complex and activation of T-cell proliferation.

**Pathway 6.** *p12I Viral protein promotes T-cell activation in HTLV-1 infection* ([Fig. 10](#)).

## Key cellular contributors and processes

### CD4+ T cell

#### Cell

CD4+ T cells are a subtype of T cells (T lymphocytes) that recognize peptides presented on the MHC class II molecules of antigen-presenting cells. CD4+ T cells protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) by stimulating B-cell maturation and the activation of other immune cells.

### Endoplasmic reticulum stress response

#### Process

The endoplasmic reticulum protein response (unfolded protein response, UPR) is a highly conserved adaptive process in eukaryotes triggered by a buildup of unfolded and/or misfolded proteins in the endoplasmic reticulum. The UPR leads to the restoration of normal cellular functioning or the elimination of severely damaged cells via apoptosis.

### Viral synapse

A viral synapse is the cell-to-cell contact that occurs between infected and noninfected cells to facilitate the transmission of viruses.

### Provirus

When viral DNA integrates to the host genome, it is called a provirus.

## Pathway 1

### HTLV-1 enters the cell (Fig. 5)

#### Incoming signals

There exist two means of HTLV entry into the cell. Mature virus particles can fuse with and be engulfed by T-cell surface membrane proteins. Alternatively, viral RNA can be passed from cell to cell via a virological synapse. HTLV-1 infects various types of cells; however, it is most effective at infecting CD4+ cells ([Matsuoka and Jeang, 2011](#)).

#### Outcome effects

As virus particles or viral RNA molecule are internalized by the cell, the virally encoded reverse transcriptase generates complementary DNA from the viral RNA genome. Further the newly synthesized viral DNA integrates into the host T cell's genome.

#### Signaling

- (a) The TM and SU proteins on the target cell's surface are key players in the process of virus entry. The virus surface envelope protein SU interacts directly with the T-cell membrane proteins neuropilin 1 (NRP1); solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1); and heparan sulfate proteoglycan ([Dutartre et al., 2016](#)).
- (b) The other mode of viral infection involves the passage of viral RNA from infected to intact CD4+ T cells through a virological synapse. The process of virological synapse formation relies on the expression of the virus protein Tax. Tat can interact with Rho GTPases; thereby regulate the activity of F-actin filaments and cytoskeleton organization; and, specifically, lead to the formation of the virological synapse ([Gross et al., 2016](#)). The LFA-1 (integrin subunit alpha L/integrin subunit beta 2) complex and intercellular adhesion molecule 1 (ICAM1) are largely responsible for the formation of this intercellular junction. The talins 1 and 2 (TLN1 and TLN2), which regulate the LFA-1 complex, are also essential to this process. In addition, the antigen polypeptide complex CD3 participates in the formation of the junction and with its interaction with the cytoskeleton. The viral genomic RNA complexed with the viral gag protein passes through the virological synapse and is internalized via endocytosis. As a result the neighboring intact T cell receives viral RNA from the infected T cell ([Dutartre et al., 2016](#)).

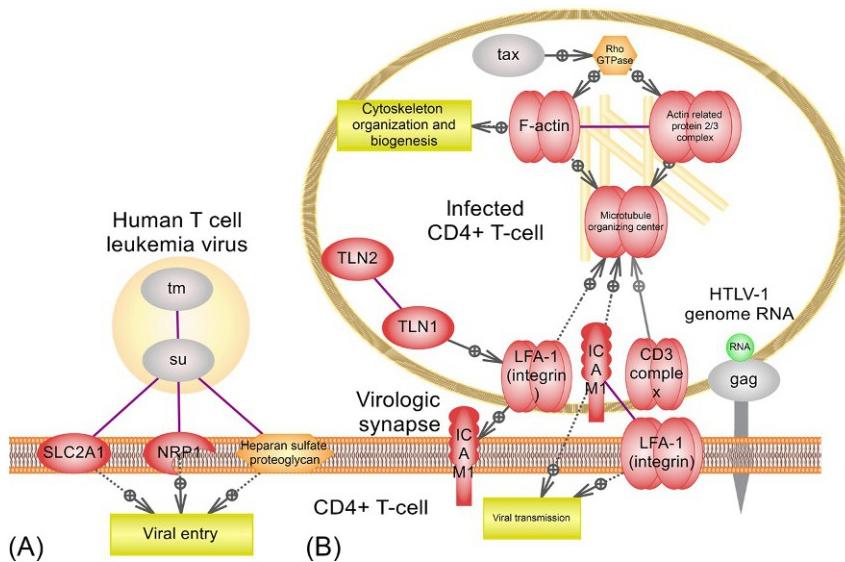


FIG. 5 Pathway 1: HTLV-1 enters the cell.

## Pathway 2

### Transcription and reproduction of the HTLV-1 genome (Fig. 6)

#### Incoming signals

The HTLV-1 genome, flanked by long terminal repeats (LTR) at 5' and 3' ends, contains several open reading frames. The *gag*, *pol*, and *env* genes encode structural proteins of the virus. The 5'LTR acts as a promoter that activates transcription of viral genes.

The *pol* ORF codes for a protein with reverse transcriptase, protease, and integrase activities, and the *gag* ORF encodes internal virion proteins. Further the *env* ORF encodes the viral envelope proteins SU and TM. Finally the HTLV-1 genomic region located between the *env* gene and 3'LTR contains ORFs that encode the tax, rex, p12, p13, p30, and p21 proteins.

Also the reverse DNA chain of the provirus is able to express the HTLV-1 basic zipper factor (HBZ) protein. The ORF encoding HBZ is located near the 3'LTR. HBZ interacts with cellular proteins, and it regulates expression of the viral tax gene ([Kannian and Green, 2010](#); [Matsuoka and Jeang, 2011](#)).

#### Outcome effects

As virion particles or viral RNA are engulfed by the host cell, structural proteins of the virus complete reverse transcription of the viral genome. The newly synthesized viral DNA then incorporates into the host genome. Expression of the virus regulatory proteins (tax, rex, p30II, p13II, and HBZ) is necessary for viral genome replication and for transcription of virus genes. However, the virus needs proteins of the host cell to complete these processes.

#### Signaling

The virus protein tax acts mainly as an activator of the LTR-dependent transcription of viral genes. Specifically, tax interacts with the cellular cAMP-responsive element-binding protein 1 (CREB1) transcription factor and the CREB-binding protein (CREBBP) protein bound to it, leading to CREB1 dimerization that increases its affinity to CRE-containing elements in promoters of many T-cell genes ([Azran et al., 2004](#)) and the viral LTRs ([Michael et al., 2006](#)). Importantly the cellular msx homeobox 2 (MSX2) and p13II proteins block tax activity ([Twizere et al., 2005](#)).

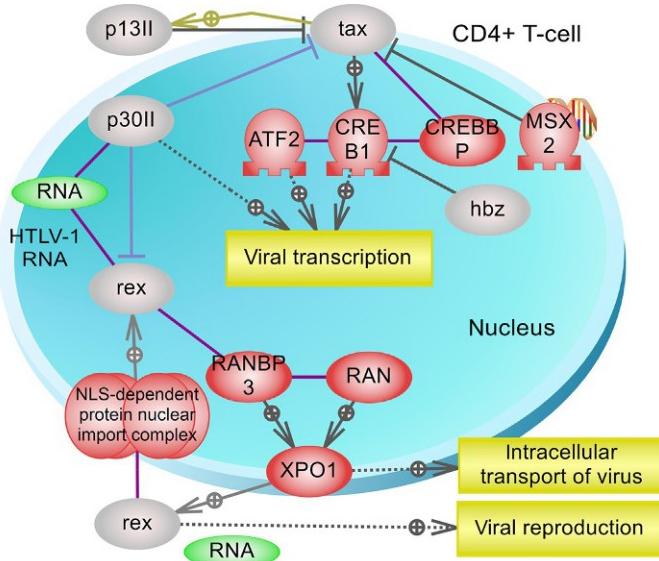
The maturation of p13II requires its posttranslational modification involving tax, which in turn increases p13II stability. As a result, stabilized

p13II is translocated into the host cell nucleus where it binds tax and hinders its connection to CREB1. This, in turn, diminishes the effectiveness of tax-induced transcription. Furthermore, another viral protein termed HBZ also interacts with CREB1 and blocks tax-dependent replication and transcription of the viral genome ([Andresen et al., 2011](#)).

The effective regulation of viral gene transcription requires increased levels of the p30II protein ([Andresen et al., 2011](#)). p30II blocks transcription of the viral tax and rex genes through binding to their respective mRNAs, thereby restricting their exit from the nucleus.

The transport of newly synthesized viral genomic RNA directly involves rex, which forms a complex with RNA. RNA-rex complexes interact with the cellular RAN-binding protein 3 (RANBP3 protein). RANBP3 is a part of the nuclear pore complex, which also includes the RAN (RAN, member RAS oncogene family) and exportin 1 (XPO1) proteins. The XPO1 protein then exports the rex-RNA complex from the nucleus into the cytoplasm where the rex protein dissociates from viral RNA.

Afterward, free rex is shuttled back into the nucleus by the NLS-dependent protein nuclear import complex, which is composed of the karyopherin subunits alpha 2 and beta 1 (KPNA2 and KPNB1) proteins ([Nakano and Watanabe, 2012](#)).



**FIG. 6** Pathway 2: Transcription and reproduction of the HTLV-1 genome.

## Pathway 3

### Tax-induced aneuploidy in T cells infected with HTLV-1 ([Fig. 7](#))

#### Incoming signals

Cell cycle progression is needed for the reproduction of virus particles. Accordingly the tax protein actively interacts with and blocks host T-cell proteins responsible for the regulation of the mitotic spindle checkpoint, vacuole 14 protein (VAC14), RAN-binding protein 1 (RANBP1), and MAX dimerization protein 1 (MXD1).

#### Outcome effects

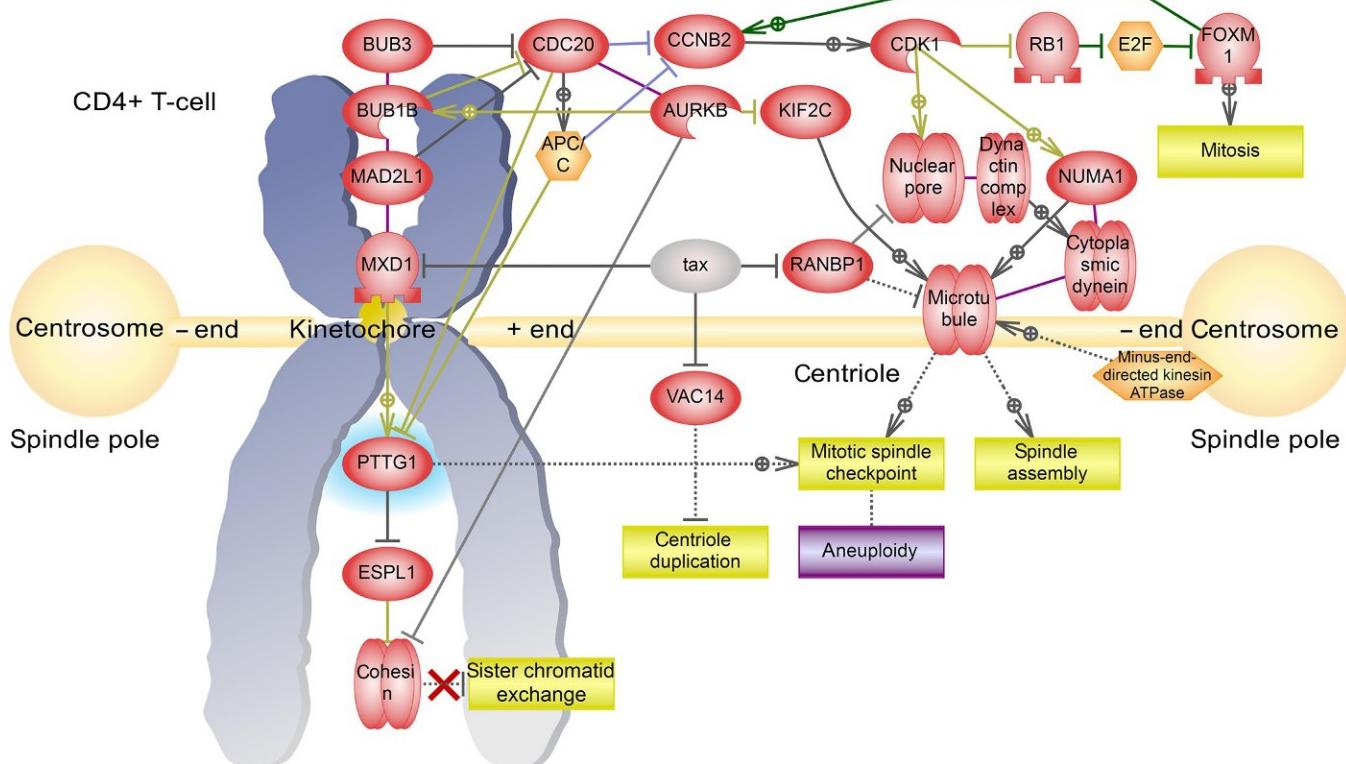
Expression of the viral tax protein ultimately disrupts the mitotic spindle checkpoint, leading to incorrect chromosome segregation, and results in aneuploidy.

#### Signalling

The HTLV-1-encoded tax protein directly blocks the activity of VAC14, RANBP1, and MXD1 by interacting with them.

The VAC14 protein is typically responsible for inhibiting centriole replication so that its binding to tax leads to centrosome hyperamplification ([Ching et al., 2006](#)). RANBP1 is involved in microtubule cytoskeleton organization. Its inhibition causes microtubule hyperstabilization and consequently disturbs mitotic spindle formation.

Finally the interaction of tax with the cellular MAX dimerization protein 1 (MXD1) protein destroys the protein complex (MXD1, MAD2L1, MAD2L1BP, BUB1B, and BUB3) responsible for the activation of spindle assembly. This event blocks the mitotic spindle checkpoint through activating cell division cycle 20 (CDC20) and anaphase-promoting complex/cyclosome (APC/C). APC/C is involved in the inactivation of major regulators of the mitotic spindle checkpoint proteins cyclin B2 (CCNB2) and pituitary tumor-transforming 1 (PTTG1) ([Boxus et al., 2008](#)). Normally the CCNB2 and PTTG1 proteins control the spindle checkpoint. Disruption of their function (through mutation or impaired activity/expression) negatively affects the spindle checkpoint transition.



**FIG. 7** Pathway 3: Tax-induced aneuploidy in T cells infected with HTLV-1.

## Pathway 4

### Tax-induced disruption of the G1/S transition in HTLV-1-infected T cells (Fig. 8)

#### Incoming signals

The HTLVs need cell cycle progression and transition through the mitotic phases for viral reproduction. The viral tax protein actively interacts with and regulates some of T-cell proteins that regulate phase changes in the cell cycle and thereby stimulate mitosis in the host cell.

#### Outcome effects

The activation of cell cycle progression diminishes the effectiveness of DNA repair, and consequently, mutations accumulate in the host cell's genome. Persistent DNA repair mechanisms and related cellular processes lead to T-cell death or transformation.

#### Signaling

The HTLV-1-encoded tax protein binds and deactivates discs large homolog 1 (DLG1), thereby inhibiting the formation of an essential signal that suppresses the cell cycle; tax blocks activation of the beta-catenin destruction complex through DLG1 ([Surena et al., 2009](#)). This activates the G1/S transition.

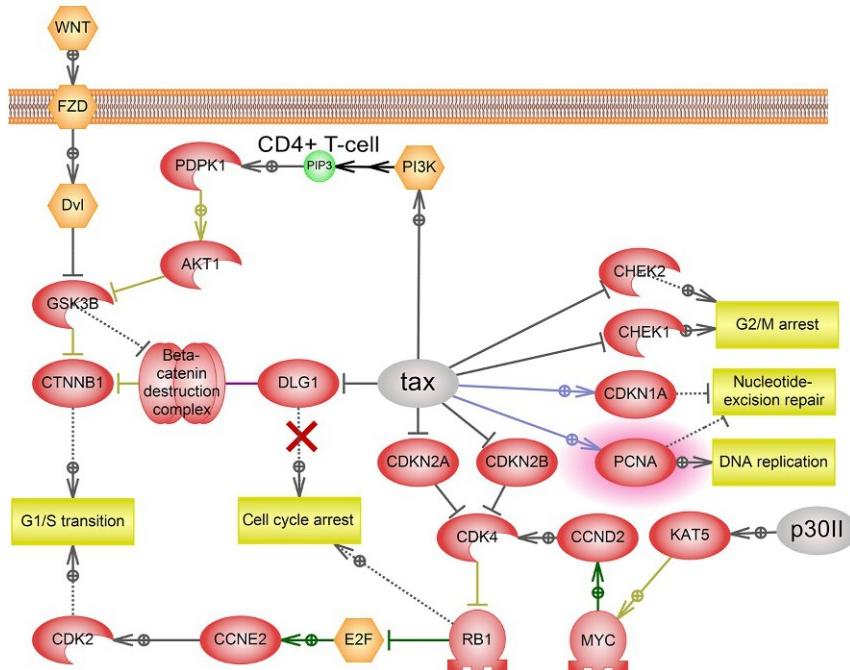
The alternative mechanism of G1/S transition activation is based on the direct interaction of tax with cyclin-dependent kinase inhibitor 2A (CDKN2A) and cyclin-dependent kinase inhibitor 2B (CDKN2B), which causes their inactivation and liberates active cyclin-dependent kinase 4 (CDK4) ([Yang et al., 2011](#)).

The viral protein p30II also participates in CDK4 activation. p30II stimulates lysine acetyltransferase 5 (KAT5), which in turn acetylates the transcription factor c-Myc proto-oncogene (MYC). In turn, MYC initiates expression of cyclin D2 (CCND2), which itself targets CDK4.

Further, activated CDK4 blocks retinoblastoma 1 (RB1), which ceases to inhibit the E2F transcription factors. The latter stimulates expression of cyclin E2 (CCNE2) and the activation of cyclin-dependent kinase 2 (CDK2). Their activation promotes the G1/S transition.

Tax activates the WNT signaling pathway acting directly through phosphatidyl inositol 3-kinase (PI3K). PI3K enhances the inhibition of glycogen synthase kinase 3 beta (GSK3B), which in turn leads to catenin beta 1 (CTNNB1) activation, therefore supporting the G1/S transition ([Olagnier et al., 2014](#)).

Moreover, tax expression targets the proliferating cell nuclear antigen (PCNA) and cyclin-dependent kinase inhibitor 1A (CDK1A) proteins, which negatively regulate the process of nucleotide excision repair (nucleotide excision repair). Overexpression of PCNA promotes DNA replication ([Boxus et al., 2008](#)). The checkpoint kinases 1 and 2 (CHEK1 and CHEK2) are also affected by tax, which binds to and inactivates them, thus blocking the inhibition of the G2/M transition ([Currey et al., 2012](#)).



**FIG. 8** Pathway 4: Tax-induced disruption of the G1/S transition in HTLV-1 infected T cell.

## Pathway 5

### Tax-mediated dysregulation of NF- $\kappa$ B signaling in HTLV-1 infection (Fig. 9)

#### Incoming signals

The HTLV-1-encoded tax protein can modify NF- $\kappa$ B signaling by interacting with many mitogen-activated protein kinase kinase kinase (MAP3K proteins) and direct interactions with one of the NF- $\kappa$ B signaling pathway proteins—*inhibitor of kappa-light polypeptide gene enhancer in B-cells, kinase gamma* (IKBKG).

#### Outcome effects

NF- $\kappa$ B signaling in CD4+ T cells is necessary for their survival. Interfering with its regulation blocks apoptosis and promotes cell survival.

#### Signaling

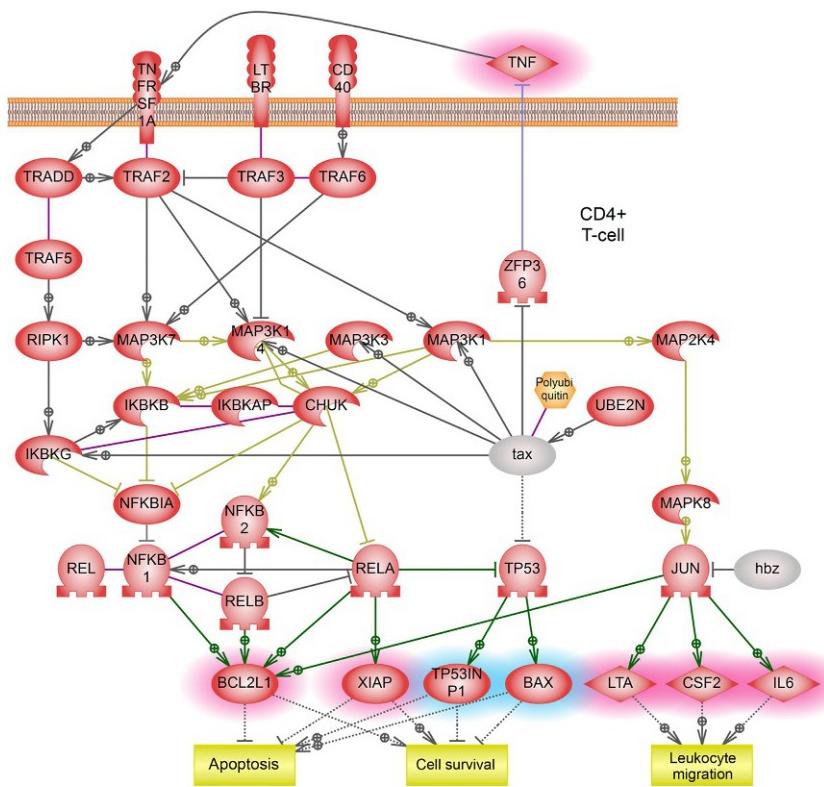
Various signals can activate the NF- $\kappa$ B signaling pathway. The tumor necrosis factor (TNF)-related activation of the mitogen-activated protein kinase kinase kinase (MAP3Ks) is a classic signal transduction route in the NF- $\kappa$ B cascade. The noncanonical activation of NF- $\kappa$ B involves the transmission of signals from MAP3K14 to conserved helix-loop-helix ubiquitinous kinase (CHUK).

The tax protein is capable of directly interacting with and subsequently activating MAP3K3, MAP3K14, and MAP3K1. However, the tax protein itself can directly regulate NF- $\kappa$ B signaling. Following UBE2N-mediated polyubiquitination, tax can bind to and activate IKBKG (Shembade et al., 2007), causing activation of the NF- $\kappa$ B complex and in turn the activation of multiple transcription factors.

Interaction of the NF- $\kappa$ B complex with tax activates expression of the XIAP (X-linked inhibitor of apoptosis, E3 ubiquitin-protein ligase) and BCL2 like 1 (BCL2L1) proteins and inhibits expression of the proapoptotic genes tumor protein p53 inducible nuclear protein 1 (TP53INP1) and BCL2-associated X protein (BAX). Consequently, apoptosis is blocked in infected T cells. Another viral protein—p13II—activates apoptosis, thereby reducing viral oncogenic potential (Andresen et al., 2011).

In addition, when activated by tax, MAP3K1 positively regulates the jun proto-oncogene (JUN) transcription factor and consequently the expression of the lymphotoxin alpha (LTA), colony-stimulating factor 2 (CSF2), and interleukin-6 (IL-6) cytokines, which subsequently activate cell proliferation. Also the expressed cytokines are responsible for

leukocyte migration. Meanwhile the virally encoded HBZ protein blocks this signaling pathway by diminishing the transcription potential of JUN ([Hivin et al., 2006](#)). The ZFP36 ring-finger protein (ZFP36) transcription factor is another target of the viral tax protein. ZPF36 negatively affects expression of the TNF ligand ([Twizere et al., 2003](#)). By blocking ZFP36 the tax protein causes overexpression of the TNF ligand and therefore stimulates the MAP3K1/JUN-signaling pathway.



**FIG. 9** Pathway 5: Tax-mediated dysregulation of NF-κB signaling in HTLV-1 infection.

## Pathway 6

### p12I viral protein promotes T-cell activation in HTLV-1 infection (Fig. 10)

#### Incoming signals

The viral protein p12I accumulates in the host cells' endoplasmic reticulum and Golgi apparatus where it interacts with its major targets, namely, the MHC class I protein complex and interleukin-2 receptor (IL-2R) complex (Cavallari et al., 2011). p12I induces MHC-I-complex degradation.

#### Outcome effects

As a result, p12I stimulates T-cell proliferation and inhibits MHC-I production. On the other hand, p12I lowers the effectiveness of NK-cell binding to the infected T cell by inhibiting IL-2R. Therefore the infected cells are not destroyed by NK cells and continue to produce mature viruses (Olière et al., 2011).

#### Signaling

In the endoplasmic reticulum the p12I protein binds the heavy chain of the MHC-I complex encoded by the HLA-2A gene (Johnson et al., 2001). The p12I-MHC-I complex activates the  $\text{Ca}^{2+}$ -dependent protein calnexin (CANX), resulting in MHC-I degradation due to its incorrect folding. Also, calreticulin (CALR) activation and the resulting calcium efflux induce the catalytic subunits of protein phosphatases (PPP3CA and PPP3CB—protein phosphatase 3 catalytic subunit alpha and beta), which in turn phosphorylate the transcription factors nuclear factors of activated T cells (NF-AT), which are responsible for the activation and proliferation of T cells (Kim et al., 2003).

In the endoplasmic reticulum, p12I binds the interleukin-2 receptor (IL-2R), which itself activates the JAK/STAT signaling pathway that is important for T-cell proliferation. At the same time, binding of p12I to IL-2R inhibits transport of the latter to the cell membrane (Mulloy et al., 1996). This prevents the activation of NK cell-mediated cytotoxicity.

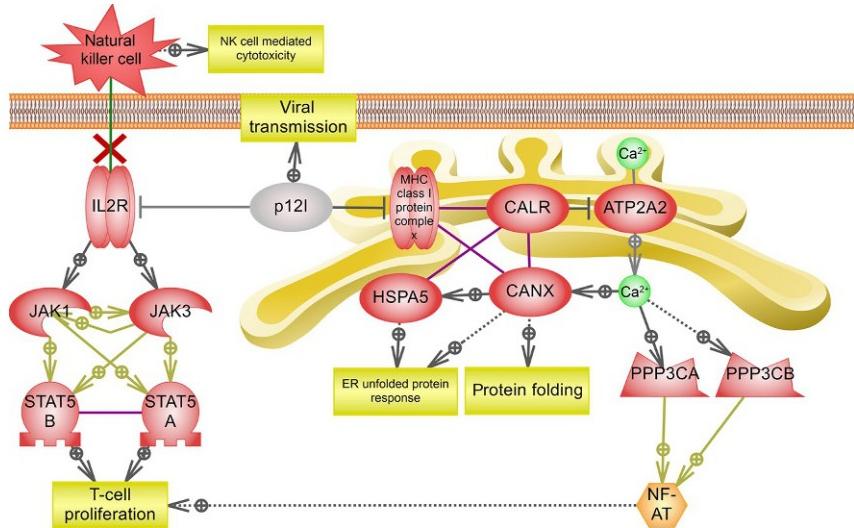


FIG. 10 Pathway 6: p12I Viral protein promotes T-cell activation in HTLV-1 infection.

## References

- Andresen, V., Pise-Masison, C.A., Sinha-Datta, U., Bellon, M., Valeri, V., Washington Parks, R., Cecchinato, V., Fukumoto, R., Nicot, C., Franchini, G., 2011. Suppression of HTLV-1 replication by Tax-mediated rerouting of the p13 viral protein to nuclear speckles. *Blood* 118, 1549–1559. <https://doi.org/10.1182/blood-2010-06-293340>.
- Azran, I., Schavinsky-Khrapunsky, Y., Aboud, M., 2004. Role of Tax protein in human T-cell leukemia virus type-I leukemogenicity. *Retrovirology* 1, 20. <https://doi.org/10.1186/1742-4690-1-20>.
- Boxus, M., Twizere, J.-C., Legros, S., Dewulf, J.-F., Kettmann, R., Willems, L., 2008. The HTLV-1 Tax interactome. *Retrovirology* 5, 76. <https://doi.org/10.1186/1742-4690-5-76>.
- Cavallari, I., Rende, F., D'Agostino, D.M., Cinimale, V., 2011. Converging strategies in expression of human complex retroviruses. *Viruses* 3, 1395–1414. <https://doi.org/10.3390/v3081395>.
- Ching, Y.-P., Chan, S.-F., Jeang, K.-T., Jin, D.-Y., 2006. The retroviral oncoprotein Tax targets the coiled-coil centrosomal protein TAX1BP2 to induce centrosome overduplication. *Nat. Cell Biol.* 8, 717–724. <https://doi.org/10.1038/ncb1432>.
- Currer, R., Van Duyne, R., Jaworski, E., Guendel, I., Sampey, G., Das, R., Narayanan, A., Kashanchi, F., 2012. HTLV tax: a fascinating multifunctional co-regulator of viral and cellular pathways. *Front. Microbiol.* 3, 406. <https://doi.org/10.3389/fmicb.2012.00406>.
- Dutartre, H., Clavière, M., Journo, C., Mahieux, R., 2016. Cell-free versus cell-to-cell infection by human immunodeficiency virus type 1 and human T-lymphotropic virus type 1: exploring the link among viral source, viral trafficking, and viral replication. *J. Virol.* 90, 7607–7617. <https://doi.org/10.1128/JVI.00407-16>.
- Gonçalves, D.U., Proietti, F.A., Ribas, J.G.R., Araújo, M.G., Pinheiro, S.R., Guedes, A.C., Carneiro-Proietti, A.B.F., 2010. Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. *Clin. Microbiol. Rev.* 23, 577–589. <https://doi.org/10.1128/CMR.00063-09>.
- Gross, C., Wiesmann, V., Millen, S., Kalmer, M., Wittenberg, T., Gettemans, J., Thoma-Kress, A.K., 2016. The Tax-inducible actin-bundling protein Fascin is crucial for release and cell-to-cell transmission of human T-cell leukemia virus type 1 (HTLV-1). *PLoS Pathog.* 12, e1005916. <https://doi.org/10.1371/journal.ppat.1005916>.
- Hivin, P., Arpin-André, C., Clerc, I., Barbeau, B., Mesnard, J.-M., 2006. A modified version of a Fos-associated cluster in HBZ affects Jun transcriptional potency. *Nucleic Acids Res.* 34, 2761–2772. <https://doi.org/10.1093/nar/gkl375>.
- Johnson, J.M., Nicot, C., Fullen, J., Cinimale, V., Casareto, L., Mulloy, J.C., Jacobson, S., Franchini, G., 2001. Free major histocompatibility complex class I heavy chain is preferentially targeted for degradation by human T-cell leukemia/lymphotropic virus type 1 p12(I) protein. *J. Virol.* 75, 6086–6094. <https://doi.org/10.1128/JVI.75.13.6086-6094.2001>.
- Kannian, P., Green, P.L., 2010. Human T lymphotropic virus type 1 (HTLV-1): molecular biology and oncogenesis. *Viruses* 2, 2037–2077. <https://doi.org/10.3390/v2092037>.
- Kim, S., Ding, W., Albrecht, B., Green, P.L., Lairmore, M.D., 2003. A conserved calcineurin-binding motif in human T lymphotropic virus type 1 p12I functions to modulate nuclear factor of activated T cell activation. *J. Biol. Chem.* 278, 15550–15557. <https://doi.org/10.1074/jbc.M210210200>.
- Matsuoka, M., Jeang, K.-T., 2011. Human T-cell leukemia virus type 1 (HTLV-1) and leukemic transformation: viral infectivity, Tax, HBZ and therapy. *Oncogene* 30, 1379–1389. <https://doi.org/10.1038/onc.2010.537>.
- Michael, B., Nair, A.M., Datta, A., Hiraragi, H., Ratner, L., Lairmore, M.D., 2006. Histone acetyltransferase (HAT) activity of p300 modulates human T lymphotropic virus type 1 p30II-mediated repression of LTR transcriptional activity. *Virology* 354, 225–239. <https://doi.org/10.1016/j.virol.2006.07.002>.

- Mulloy, J.C., Crownley, R.W., Fullen, J., Leonard, W.J., Franchini, G., 1996. The human T-cell leukemia/lymphotropic virus type 1 p12I proteins bind the interleukin-2 receptor beta and gamma chains and affects their expression on the cell surface. *J. Virol.* 70, 3599–3605.
- Nakano, K., Watanabe, T., 2012. HTLV-1 Rex: the courier of viral messages making use of the host vehicle. *Front. Microbiol.* 3, 330. <https://doi.org/10.3389/fmicb.2012.00330>.
- Olagnier, D., Sze, A., Bel Hadj, S., Chiang, C., Steel, C., Han, X., Routy, J.-P., Lin, R., Hiscott, J., van Grevenynghe, J., 2014. HTLV-1 Tax-mediated inhibition of FOXO3a activity is critical for the persistence of terminally differentiated CD4+ T cells. *PLoS Pathog.* 10, e1004575. <https://doi.org/10.1371/journal.ppat.1004575>.
- Olière, S., Douville, R., Sze, A., Belgnaoui, S.M., Hiscott, J., 2011. Modulation of innate immune responses during human T-cell leukemia virus (HTLV-1) pathogenesis. *Cytokine Growth Factor Rev.* 22, 197–210. <https://doi.org/10.1016/j.cytofr.2011.08.002>.
- Shembade, N., Harhaj, N.S., Yamamoto, M., Akira, S., Harhaj, E.W., 2007. The human T-cell leukemia virus type 1 Tax oncoprotein requires the ubiquitin-conjugating enzyme Ubc13 for NF-kappaB activation. *J. Virol.* 81, 13735–13742. <https://doi.org/10.1128/JVI.01790-07>.
- Surena, A.-L., de Faria, G.P., Studler, J.-M., Peiretti, F., Pidoux, M., Camonis, J., Chneiweiss, H., Formstecher, E., Junier, M.-P., 2009. DLG1/SAP97 modulates transforming growth factor alpha bioavailability. *Biochim. Biophys. Acta* 1793, 264–272. <https://doi.org/10.1016/j.bbamcr.2008.09.005>.
- Twizere, J.-C., Kruys, V., Lefebvre, L., Vanderplasschen, A., Collete, D., Debacq, C., Lai, W.S., Jauniaux, J.-C., Bernstein, L.R., Semmes, O.J., Burny, A., Blackshear, P.J., Kettmann, R., Willem, L., 2003. Interaction of retroviral Tax oncoproteins with tristetraprolin and regulation of tumor necrosis factor-alpha expression. *J. Natl. Cancer Inst.* 95, 1846–1859.
- Twizere, J.-C., Lefèuvre, L., Collete, D., Debacq, C., Urbain, P., Heremans, H., Jauniaux, J.-C., Burny, A., Willem, L., Kettmann, R., 2005. The homeobox protein MSX2 interacts with tax oncoproteins and represses their transactivation activity. *J. Biol. Chem.* 280, 29804–29811. <https://doi.org/10.1074/jbc.M503674200>.
- Yang, L., Kotomura, N., Ho, Y.-K., Zhi, H., Bixler, S., Schell, M.J., Giam, C.-Z., 2011. Complex cell cycle abnormalities caused by human T-lymphotropic virus type 1 Tax. *J. Virol.* 85, 3001–3009. <https://doi.org/10.1128/JVI.00086-10>.

## CHAPTER

## 2.3

## Human papillomavirus infection and cancer

Human papillomavirus (HPV) is a DNA-containing virus capable of infecting basal epithelial cells of the skin and squamous epithelium cells. Human papillomavirus infection is manifested by the appearance of neoplasms of the skin and mucous membranes (papilloma), which can transform into malignant tumors.

Currently, more than 100 types of HPV have been discovered. Certain types of HPV can infect a strictly defined type of epithelium. Different types of HPV exhibit different activity regarding the transformation of papillomas into tumors. There are low-risk HPV types, for example, types 6, 11, 42, 43, and 44, and high-risk types, for example, types 16, 18, 31, 33, and 45. HPV16 infects cervical epithelium and is the main cause of cervical cancer.

The known HPV types are similar in their genetic structure. The genetic material of the virus is represented by a circular double-stranded DNA molecule 7200–8000 base pair long. One DNA strand contains eight open reading frames that encode eight proteins and a regulatory genomic region (URR—upstream regulatory region). The other strand is noncoding. The virus uses cellular proteins to replicate itself.

The penetration of HPVs into the cell occurs through the interaction of viral envelope proteins with the membrane proteins of the host cell.

**Pathway 1.** *Human papillomavirus entry into the cell* (Fig. 11).

The open reading frames of the viral genome are divided into early (E) and late (L) regions. Transcription of viral DNA proceeds sequentially through the E1, E2, E4 and E5, E6, and E7 genes.

The E1 and E2 viral proteins are required for replication of the virus, cell cycle activation, and maintenance of the nononcogenic status of the infected cell.

**Pathway 2.** *Human papillomavirus E1 and E2 proteins in virus replication* (Fig. 12).

Proteins E4 and E5 of the virus are necessary for maturation of viral particles.

**Pathway 3.** *Human papillomavirus E4 and E5 proteins in viral reproduction* (Fig. 13).

Expression of oncogenic viral proteins E6 and E7 leads to a block of apoptosis and cstimulation of cell proliferation. In some cases, overexpression of E6 and E7 can promote oncogenesis.

**Pathway 4.** *Human papillomavirus E6 and E7 proteins promote epithelial cell survival (Fig. 14).*

**Pathway 5.** *Human papillomavirus E6 and E7 proteins promote epithelial cell proliferation (Fig. 15).*

## Key cellular contributors and processes

Episomal form

Process

The episomal form of a virus refers to a latent state in which the virus does not integrate into the host genome and exists as an episome inside the host cell's nucleus.

Nuclear pore complex

Anatomic structure

Nuclear pore complexes are openings in the nuclear envelope that allow for the highly selective transport of various substances in and out of the nucleus.

Viral genome encapsidation

Process

Viral genome encapsidation is the process of enclosing a viral DNA or RNA genome into a protein capsid.

## Pathway 1

### Human papillomavirus entry into cell (Fig. 11)

#### Incoming signals

The HPV reaches the basal layer of the epithelium through micro-trauma of overlying cells. The initial stage of the infection involves entry of viral particles into the cell and transport of viral DNA into the nucleus. In the early stages of infection, viral DNA is a circular molecule in extrachromosomal or episomal form.

#### Outcome effects

The internalization of viral particles into a host cell and the translocation of viral DNA into the nucleus lead to replication of the viral genome and transcriptional activation of viral genes.

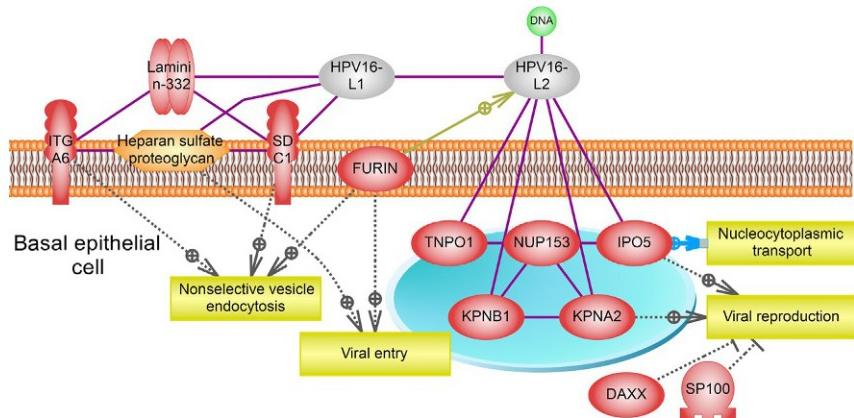
#### Signaling

Capsid proteins of HPVs encase and protect the viral nucleic acid genome, providing the initial interaction site of the viral particle with the host cell. The HPV genome is surrounded by an icosahedral capsid consisting of two structural proteins: the major capsid protein L1 (HPV16-L1) and the minor capsid protein L2 (HPV16-L2). The L1 proteins are highly conserved and aggregate to form 72 fivefold capsomers. The L2 protein binds viral DNA. It is an internal multifunctional protein with roles in genome encapsidation, capsid stabilization, endosomal escape of virions, and nuclear transport of the HPV genome.

The initial binding of the virus to the cell surface is mediated by L1, which interacts with heparan sulfate proteoglycans (HSPGs) present on the host cell ([Ahasan et al., 2015](#)). L1 can also bind to the laminin-332 complex and syndecan-1 (SDC1). Following receptor engagement by capsid proteins, the virus is internalized, and its coat is disassembled. Proteolytic cleavage of L2 by FURIN is the next essential step for successful infection. Internalization of the virus can occur via different routes including clathrin-, caveolae-, and lipid raft-dependent or independent mechanisms. After entering the cell, L2 transfers the viral genome to the nucleus where it accumulates at the ND-10 domain (PML bodies). It is assumed that the components of ND-10, such as the DAXX and SP100 proteins, repress viral gene expression and protect the host cell.

On the nuclear membrane, L2 can interact with karyopherins or importins including importin 5 (IPO5), transportin-1 (TNPO1), and karyopherins KPNB1 and KPNA2. Karyopherins mediate viral DNA entering the

nucleus. Specifically the karyopherin beta and importin beta protein superfamilies interact with nucleoporins of the nuclear pore complex to transport the proteins into the nucleus (Horvath et al., 2010; Klucavsek et al., 2006; Wang and Roden, 2013).



**FIG. 11** Pathway 1: Human papillomavirus entry into the cell.

## Pathway 2

### Human papillomavirus E1 and E2 proteins in virus replication ([Fig. 12](#))

#### Incoming signals

Once the episomal viral genome penetrates the nucleus of the host cell, transcription of the early viral genes begins. HPV expresses the E1 (HPV16-E1) and E2 (HPV16-E2) proteins in the initial stage of infection.

#### Outcome effects

The E1 (HPV16-E1) and E2 (HPV16-E2) proteins activate viral DNA replication, and they promote epithelial cell proliferation.

The viral genome can exist in the host cell in two forms: as episomes and integrated into the host genome. The open reading frames of the viral genome are divided into early (E) and late (L) regions. Transcription of the episomal viral DNA is initiated in the upstream regulatory region (URR) and proceeds sequentially through the E6, E7, E1, E2, E4, and E5 genes. Transcription is terminated at the end of the entire early genome region at the polyA addition site located at the end of the E5 gene.

If the viral genome is integrated into the host cell's genome, its transcription is also initiated in URR, but it continues through the E6 and E7 genes and through part of the E1+E2 region, and then, it continues into the adjacent cellular sequences and terminates at the cellular transcription termination site closest to the 3' end of the viral DNA. Thus, following integration, expression of the E2 gene is lost, and E6 and E7 gene expression is preserved ([Klimov, 2010](#)).

#### Signaling

The HPV16-E1 protein binds to and recruits cellular replication proteins to the viral origin of replication. Those proteins include the alpha DNA polymerase-primase, replication factor A, and human topoisomerase I (TOP1). HPV16-E1 and HPV16-E2 interact with DNA directly by binding to the origin of replication in the HPV genome. Then, HPV16-E2 is displaced, and HPV16-E1 is converted to a double hexamer that unwinds the DNA in an ATP-dependent manner.

The HPV-E2 protein may interact and block the function of the cell division cycle protein 20 homolog (CDC20) and cadherin 1 (CDH1). Inhibition of CDC20 and CDH1 results in the rescue of ubiquitination of cyclin B2 (CCNB2) and aurora kinase A (AURKA) by the anaphase-promoting

complex/cyclosome (APC/C) ubiquitin ligase. Uninhibited AURKA causes excessive centrosome duplication and genomic instability.

HPV16-E2-mediated inhibition of APC/C also stabilizes the S-phase kinase-associated protein 2 (SKP2) before the cells reach the G1/S transition. SKP2 is an E3 ubiquitin-protein ligase that can degrade HPV-E2 via the SCF/SKP2 complex. The HPV16-E2 protein triggers the accumulation of SKP2, which in turn can degrade HPV16-E2. This is an example of negative feedback between viral and host cell proteins.

Maximum expression of the HPV16-E2 protein is observed in the G1 phase of the cell cycle, and it then declines because of degradation by the SCF/SKP2 complex in late G1 phase.

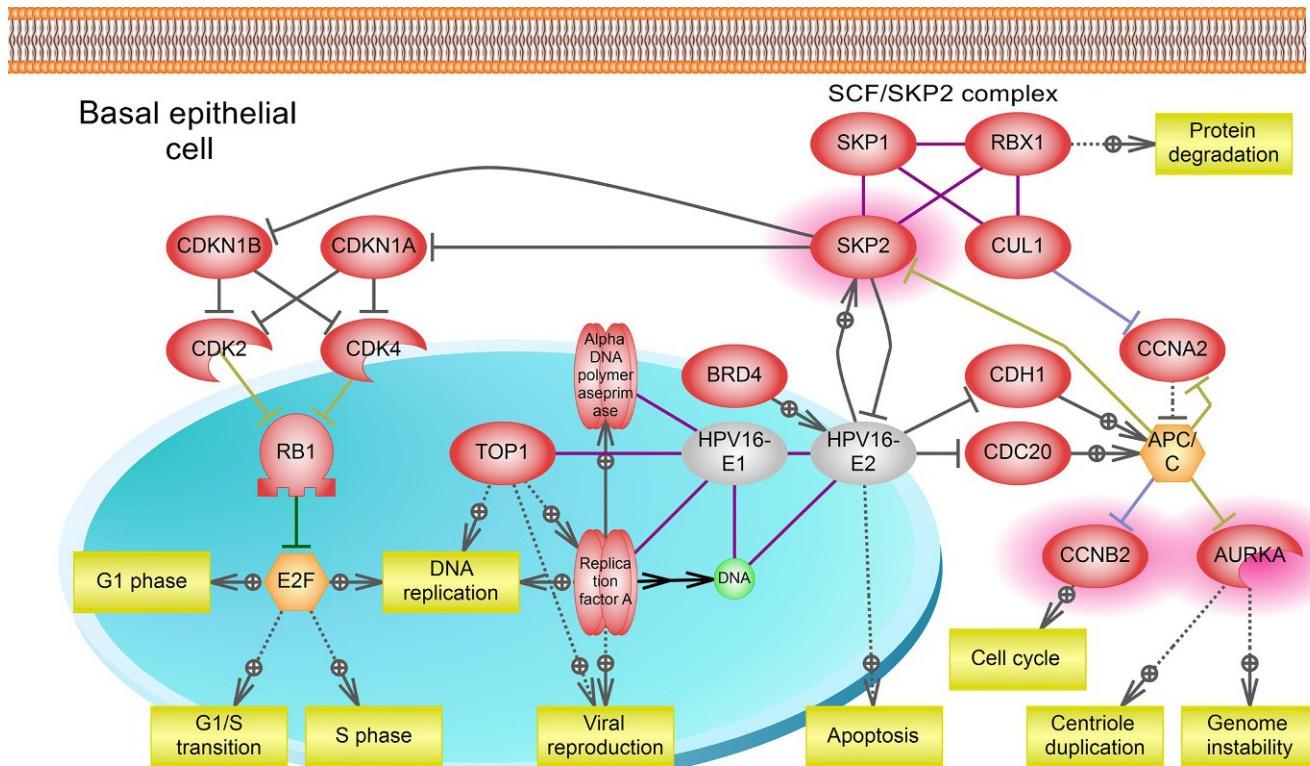
The SKP1/Cullin1/F box (SCF) is a ring-finger ubiquitin ligase composed of four subunits, which include three invariable components (RING-box protein 1 (RBX1), cullin1, and S-phase kinase-associated protein 1 (SKP1)) and one variable component, namely, an F-box protein, such as SKP2, which directly binds the substrates.

The interaction of HPV16-E2 with bromodomain containing 4 (BRD4) blocks HPV16-E2 ubiquitination.

The HPV16-E2 protein accumulates in the granular layer of HPV-associated cervical intraepithelial neoplasia, and it is not detected in proliferating cells of the basal layer. This is probably because differentiated cells, but not proliferating cells, express SKP2.

Very small amounts of HPV16-E2 can inhibit APC/C and thus stabilize SKP2, whereas higher levels of HPV16-E2 induce apoptosis (Bellanger et al., 2010; Cai et al., 2013; Chojnacki and Melendy, 2018; Doorbar, 2006; McKinney et al., 2015; Mo et al., 2012).

## II. Human disease pathways



**FIG. 12** Pathway 2: Human papillomavirus E1 and E2 proteins in virus replication.

## Pathway 3

### Human papillomavirus E4 and E5 proteins in viral reproduction ([Fig. 13](#))

#### Incoming signals

The HPV16-E5 protein is expressed during the early phases of viral reproduction. The open reading frame (ORF) of the papillomavirus HPV16-E4 gene is located within the ORF of the E2 gene. The HPV16-E5 open reading frame (ORF) has been classified into four different groups: alpha, beta, gamma, and delta, which correlate with different clinical manifestations and differing oncogenic potentials of the HPV16-E5 proteins. The HPV16-E5 ORF may be completely absent from many HPV genomes and may explain why not all HPV types are oncogenic.

#### Outcome effects

The expression of viral proteins E4 (HPV16-E4) and E5 (HPV16-E5) is required for the maturation of viral particles and their subsequent exit from the cell. Expression of HPV16-E5 protein is important for the transport of viral capsid proteins, viral genomic DNA, and the assembly of virus particles. The activity of the HPV16-E4 protein is necessary for the destruction of keratin (due to the deposition of amyloid fibrils) and the release of viral particles from basal epithelial cells.

HPV16-E5 protein expression leads to the G1/S cell cycle transition in the epithelial cell, the block of apoptosis, and the activation of angiogenesis. Since stimulation of cell proliferation and angiogenesis are significant factors in the initial stages of tumorigenesis, HPV16-E5 expression may have oncogenic effects.

#### Signaling

HPV16-E5 is a small (about 85 amino acid) extremely hydrophobic protein membrane protein. HPV16-E5 is present in the endoplasmic reticulum, nuclear envelope, Golgi apparatus, and, when overexpressed, on the plasma membrane.

By binding and inhibiting ATPase H<sup>+</sup> transporting V0 subunit c (ATP6V0C) and by interacting with karyopherin beta 3 (IPO5), HPV16-E5 affects intracellular vesicle trafficking. The inhibition of ATP6V0C also blocks apoptosis by downregulating CASP3 and CASP7 (caspase-3 and caspase-7)—key proteins of the extrinsic apoptotic pathway.

Endosome transport influences the activation of cellular cascades, which in turn induce cell proliferation while concurrently stimulating the

transcription of viral genes, including the oncogenic E6 and E7 proteins (see [Pathway 4](#)).

HPV16-E5 hampers acidification and degradation of epidermal growth factor receptor (EGFR) and endothelin receptor type A (EDNRA) by interfering with membrane trafficking and the fusion of early and late endosomes. Thus HPV infection increases the recycling levels of these receptors to the plasma membrane following stimulation of the EGFR and EDNRA signaling cascades.

Stimulation of the EGFR signaling pathway results in the activation of the transcription factors FOS-JUN (FBJ murine osteosarcoma viral oncogene homolog and transcription factor AP-1 complex) and MYC (myelocytomatosis viral oncogene homolog), thereby forcing cells to progress through the cell cycle.

EGFR signaling also activates the transcription factors the cAMP-responsive element-binding protein 1 (CREB1) and hypoxia inducible factor 1 alpha subunit (HIF1A). CREB1 initiates the transcription of B-cell CLL/lymphoma 2 protein (BCL2) that in turn blocks apoptosis and also, along with HIF1A, initiates the transcription of vascular endothelial growth factor A (VEGF) that in turn induces angiogenesis ([DiMaio and Mattoon, 2001](#); [Doorbar, 2006](#); [Venuti et al., 2011](#)).

In addition, HPV16-E5 disrupts the gap junction protein (GJA1), which is necessary for intracellular communication at gap junctions and for supporting the formation of intercellular viral synapses.

The HPV16-E4 protein is detected during amplification of the viral genome, and it is present in greatest amounts at the late stage of virus replication, before assembly and release of viral particles. This protein contributes to the process of viral genome replication, and it promotes the release of viral particles from the host cell. Phosphorylation of HPV16-E4 by mitogen-activated protein kinase 1 (MAPK1) stabilizes HPV16-E4.

HPV16-E4 directly binds to type 1 keratins (e.g., keratin 18) but not type 2 keratins or vimentin. Binding is dependent on 16 amino acids at the N-terminus of the HPV16-E4 protein, with the leucine cluster therein being required for binding.

The protease calpain cleaves HPV16-E4 after amino acid residue number 17 to generate C-terminal fragments that lack the N-terminus. These C-terminal fragments can polymerize to form amyloid-like fibers. This event can lead to the accumulation of HPV16-E4 and disrupt the normal dynamics of keratin networks in the infected tissue.

HPV16-E4 is also phosphorylated by the cyclin-dependent kinases (CDKs, cyclin B1/CDK1, and cyclin A2/CDK2 complexes) during the G2-phase of the cell cycle. Recruited HPV16-E4 may arrest cell cycle progression by preventing of CDK1/cyclin B1 complexes from entering the nucleus ([Doorbar, 2006, 2013](#); [Wang et al., 2004](#)).

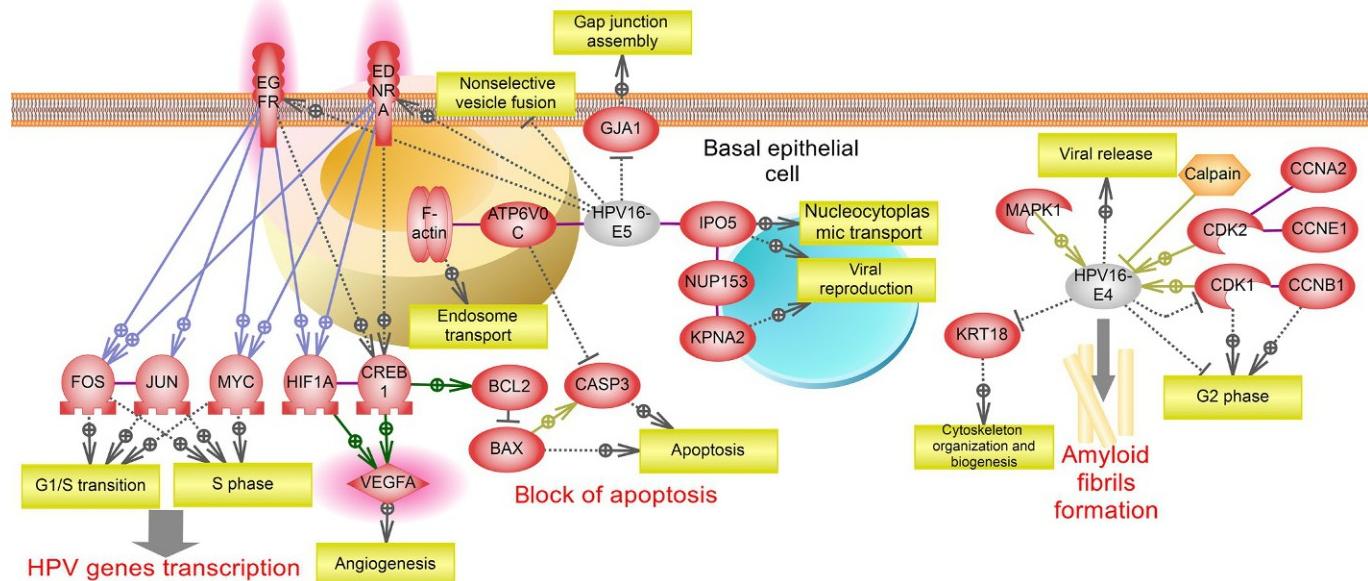


FIG. 13 Pathway 3: Human papillomavirus E4 and E5 proteins in viral reproduction.

## Pathway 4

### Human papillomavirus E6 and E7 proteins promote epithelial cell survival (Fig. 14)

#### Incoming signals

The E6 (HPV16-E6) and E7 (HPV16-E7) proteins are early viral proteins that are required for cellular transformation and the production of new viral particles.

The HPV-E2 protein binds directly to the viral HPV16-E6 and HPV16-E7 promoters to inhibit their transcription. In 62% of late-stage cervical carcinomas associated with high-risk types of HPV, integration of viral DNA into the host genome disrupts the HPV-E2 open reading frame resulting in uncontrolled HPV16-E6 and HPV16-E7 expression ([Vinokurova et al., 2008](#)).

#### Outcome effects

The HPV16-E6 and HPV16-E7 proteins inhibit the tumor suppressors tumor protein p53 (TP53) and retinoblastoma protein 1 (RB1) and the nuclear transcription factor (NFX1), thus stimulating host cell survival and viral reproduction.

The uninhibited expression of HPV16-E6 and HPV16-E7 proteins leads to the transformation of papillomas into malignant tumors.

#### Signaling

The E6 protein interacts with and activates the cellular ubiquitin-protein ligase E3A (UBE3A). In turn, UBE3A deactivates, via ubiquitination, the main targets of the viral protein E6, namely, TP53, and NFX1.

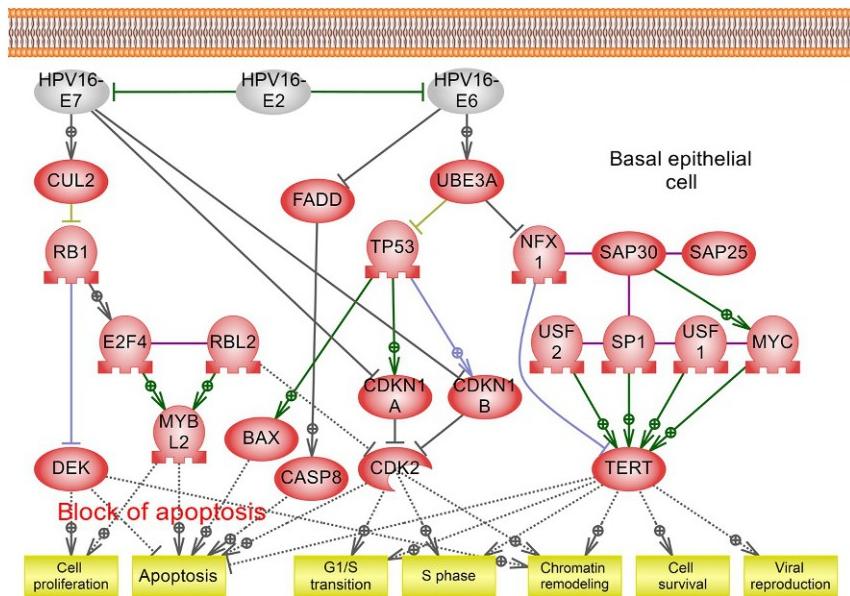
NFX1 interacts with SAP30 and SAP25 (Sin3A associated protein 30 kDa and 25 kDa, respectively), which in turn represses transcription of the cellular telomerase reverse transcriptase (TERT) gene. TERT regulates a multitude of cellular processes. When NFX1 does not repress TERT, the cell cycle progresses from G1 phase to S phase. The telomerase activity of TERT leads to chromatin remodeling, cell survival, and viral reproduction. Also, activation of TERT blocks apoptosis.

Downregulated TP53 is unable to activate the cyclin-dependent kinase inhibitors (CDKN1A and CDKN1B). This leads to activation of the cyclin-dependent kinase 2 (CDK2) and stimulation of the G1-S transition. Also, suppressed TP53 does not activate transcription of the BCL2-associated X protein (BAX) gene, which is essential for apoptosis in normal cells.

Additionally the E6 protein causes degradation of FADD, with the loss of cellular FADD proportional to the amount of E6 expressed. The E7 viral protein negatively interacts with the cyclin-dependent kinase inhibitors (CDKN1A and CDKN1B) leading to the activation of CDK2.

The primary target of the viral protein HPV16-E7 is RB1. Downregulation of RB1 by HPV is achieved through activation of cullin 2 (CUL2), which is one of the ubiquitination complex proteins. HPV16-E7 destabilizes RB1 levels through CUL2-mediated proteasomal degradation. In the absence of RB1, the DEK oncogene is activated leading to a block of apoptosis and promotion of cell proliferation.

Besides, in HIV-infected cells, there is no interaction between RB1 and the E2F transcription factor 4 (E2F4)/retinoblastoma-like 2 (RBL2). Consequently, MYB proto-oncogene like 2 (MYBL2) is not activated. MYBL2 is a transcription factor implicated in cell cycle regulation, apoptosis, and cancer (Cai et al., 2013; Chung and Gillison, 2009; Doorbar, 2006; Halim et al., 2013; Howie et al., 2009; Liu et al., 2008).



**FIG. 14** Pathway 4: Human papillomavirus E6 and E7 proteins promote epithelial cell survival.

## Pathway 5

### Human papillomavirus E6 and E7 proteins promote epithelial cell proliferation ([Fig. 15](#))

#### Incoming signals

CpG islands in the viral DNA genome are ligands for endosomal receptors like the toll-like receptor TLR9. Viral reproduction leads to the activation of TLRs and the tumor necrosis factor (TNF) pathways, which usually cause apoptosis of infected cells through receptor interacting protein (RIPK) and Fas-associated via death domain (FADD) signaling.

While the uncontrolled expression of HPV16-E6 and HPV16-E7 proteins may block apoptosis and activate cell survival pathways through parallel parts of TLRs and TNF cascades.

#### Outcome effects

When transcriptional control of the HPV16-E6 and HPV16-E7 proteins is lost because of the viral HPV-E2 protein, overexpression of nuclear factor kappaB (NF- $\kappa$ B) stimulated target genes leads to cell survival. HPV16-E6 and HPV16-E7 at the same time block apoptosis and the intracellular sensing of the viral DNA. NF- $\kappa$ B-dependent proliferation and protection from apoptosis together facilitate the survival of infected cells and the promotion of viral reproduction, which are both significant steps in the pathogenesis of HPV-related diseases.

#### Signaling

The viral proteins HPV16-E6 and HPV16-E7 can block expression of the toll-like receptor 9 (TLR9) protein. This weakens the cellular response to viral infection. Additionally the E6-mediated degradation of FADD prevents the transmission of apoptotic signals from TLR9/TNF pathways. Specifically the activation of caspase-8 by FADD does not occur in the presence of the viral protein HPV16-E6.

However, HPV can maintain the TLR9/TNF-mediated NF- $\kappa$ B activation. In the course of the TLR-mediated cellular response to the virus, in addition to FADD, TNF receptor-associated factor 6 (TRAF6) is activated. Increased activity of TRAF6 and other members of the cascade result in NFKB inhibitor alpha (NFKBIA) phosphorylation. In parallel, activation of CUL1/SKP1/BTRC/RBX1 complex by the viral HPV-E2 protein occurs. This complex activates the ubiquitination of phosphorylated NFKBIA. Degradation of NFKBIA leads to the nuclear translocation of NF- $\kappa$ B and the subsequent transcription of its target genes.

NF- $\kappa$ B activation leads to the transcription of cytokines, activators of vascularization, negative regulators of apoptosis, cyclin D1, MYC, and the FADD-like apoptosis regulator (CFLAR). The CFLAR protein can inhibit both FADD and caspase-8 (CASP8). Taken together, these events lead to a block of apoptosis, genome instability, cell survival, and vascularization of transformed tissues (Beaudenon and Huibregtse, 2008; Deligeoroglou et al., 2013; Doorbar, 2006; Howie et al., 2009; Zhou et al., 2011).

## II. Human disease pathways

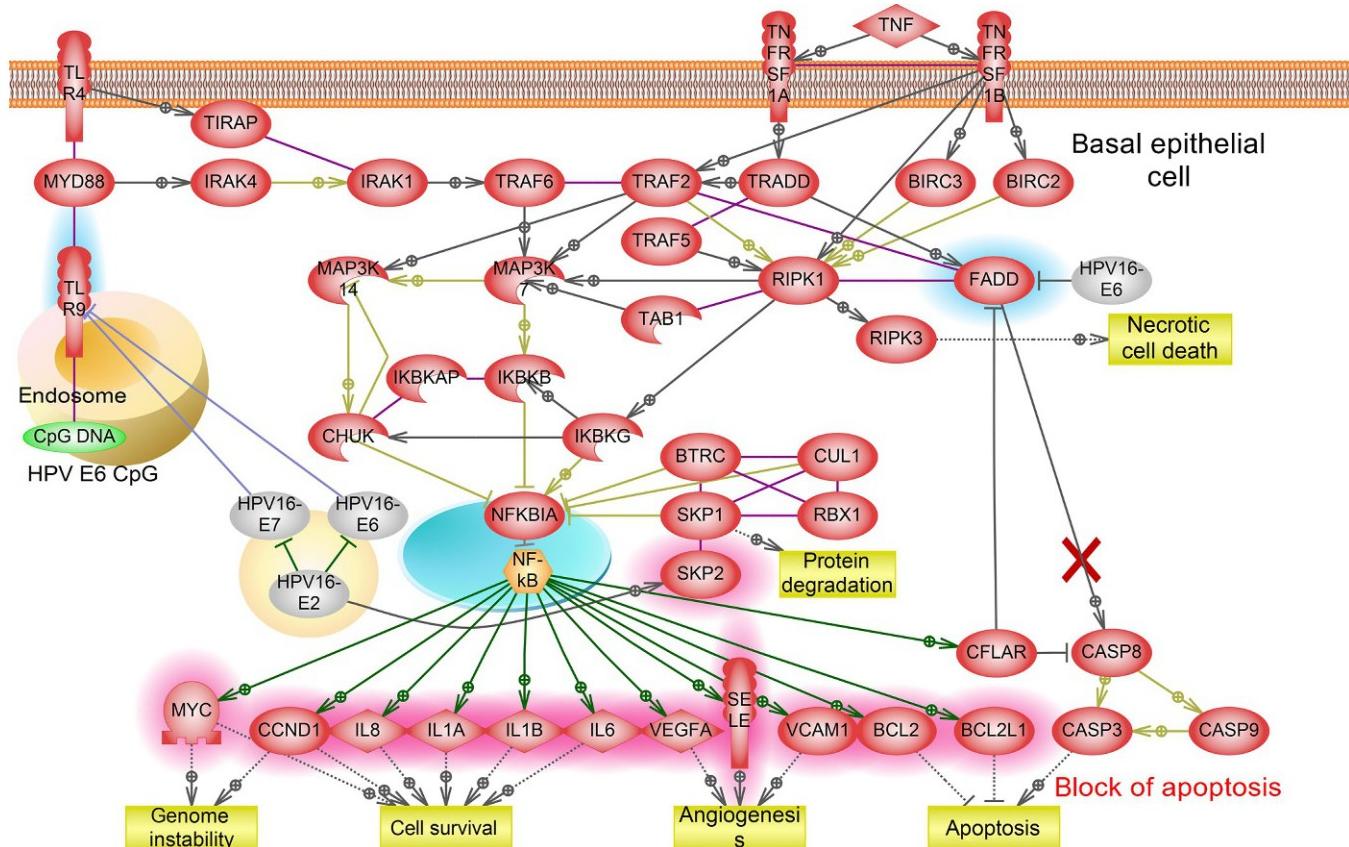


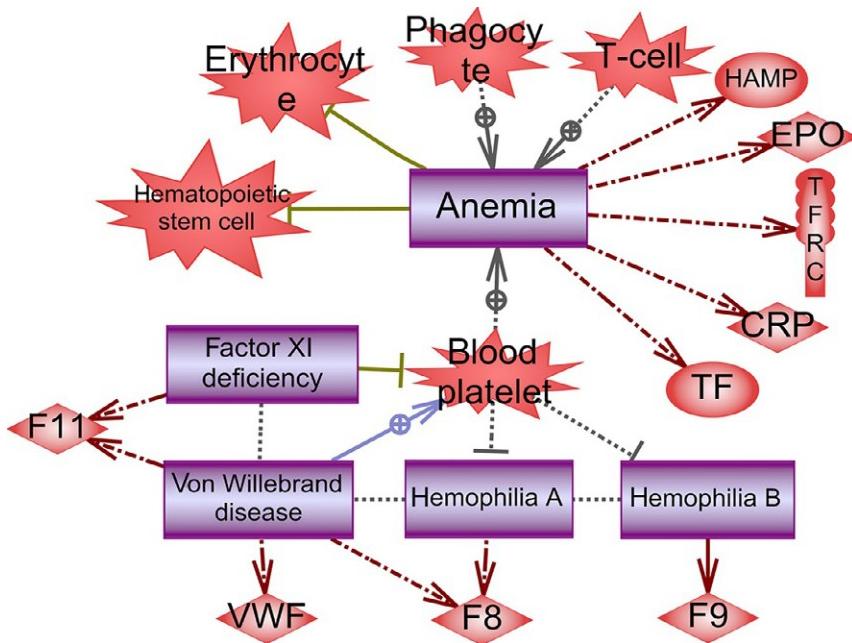
FIG. 15 Pathway 5: Human papillomavirus E6 and E7 proteins promote epithelial cell proliferation.

## References

- Disease number # 167960, # 600762, # [604461](#) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- Ahasan, M.M., Wakae, K., Wang, Z., Kitamura, K., Liu, G., Koura, M., Imayasu, M., Sakamoto, N., Hanaoka, K., Nakamura, M., Kyo, S., Kondo, S., Fujiwara, H., Yoshizaki, T., Mori, S., Kukimoto, I., Muramatsu, M., 2015. APOBEC3A and 3C decrease human papillomavirus 16 pseudovirion infectivity. *Biochem. Biophys. Res. Commun.* 457, 295–299. <https://doi.org/10.1016/j.bbrc.2014.12.103>.
- Beaudenon, S., Huibregtse, J.M., 2008. HPV E6, E6AP and cervical cancer. *BMC Biochem.* 9 (Suppl. 1), S4. <https://doi.org/10.1186/1471-2091-9-S1-S4>.
- Bellanger, S., Tan, C.L., Nei, W., He, P.P., Thierry, F., 2010. The human papillomavirus type 18 E2 protein is a cell cycle-dependent target of the SCFSkp2 ubiquitin ligase. *J. Virol.* 84, 437–444. <https://doi.org/10.1128/JVI.01162-09>.
- Cai, Q., Lv, L., Shao, Q., Li, X., Dian, A., 2013. Human papillomavirus early proteins and apoptosis. *Arch. Gynecol. Obstet.* 287, 541–548. <https://doi.org/10.1007/s00404-012-2665-z>.
- Chojnacki, M., Melendy, T., 2018. The human papillomavirus DNA helicase E1 binds, stimulates, and confers processivity to cellular DNA polymerase epsilon. *Nucleic Acids Res.* 46, 229–241. <https://doi.org/10.1093/nar/gkx1103>.
- Chung, C.H., Gillison, M.L., 2009. Human papillomavirus in head and neck cancer: its role in pathogenesis and clinical implications. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 15, 6758–6762. <https://doi.org/10.1158/1078-0432.CCR-09-0784>.
- Deligeorgoglou, E., Giannouli, A., Athanasopoulos, N., Karountzos, V., Vatopoulou, A., Dimopoulos, K., Creatsas, G., 2013. HPV infection: immunological aspects and their utility in future therapy. *Infect. Dis. Obstet. Gynecol.* 2013, 540850. <https://doi.org/10.1155/2013/540850>.
- DiMaio, D., Mattoon, D., 2001. Mechanisms of cell transformation by papillomavirus E5 proteins. *Oncogene* 20, 7866–7873. <https://doi.org/10.1038/sj.onc.1204915>.
- Doorbar, J., 2006. Molecular biology of human papillomavirus infection and cervical cancer. *Clin. Sci.* 110, 525–541. <https://doi.org/10.1042/CS20050369>.
- Doorbar, J., 2013. The E4 protein; structure, function and patterns of expression. *Virology* 445, 80–98. <https://doi.org/10.1016/j.virol.2013.07.008>.
- Halim, T.A., Farooqi, A.A., Zaman, F., 2013. Nip the HPV encoded evil in the cancer bud: HPV reshapes TRAILS and signaling landscapes. *Cancer Cell Int.* 13, 61. <https://doi.org/10.1186/1475-2867-13-61>.
- Horvath, C.A.J., Boulet, G.A.V., Renoux, V.M., Delvenne, P.O., Bogers, J.-P.J., 2010. Mechanisms of cell entry by human papillomaviruses: an overview. *Virol. J.* 7, 11. <https://doi.org/10.1186/1743-422X-7-11>.
- Howie, H.L., Katzenellenbogen, R.A., Galloway, D.A., 2009. Papillomavirus E6 proteins. *Virology* 384, 324–334. <https://doi.org/10.1016/j.virol.2008.11.017>.
- Klimov, E., 2010. Integration of human papilloma viruses into host cell genome and pathogenesis of cervical cancer. *Uspekhi Sovrem. Biol.* 130, 381–389.
- Klucevsek, K., Daley, J., Darshan, M.S., Bordeaux, J., Moroianu, J., 2006. Nuclear import strategies of high-risk HPV18 L2 minor capsid protein. *Virology* 352, 200–208. <https://doi.org/10.1016/j.virol.2006.04.007>.
- Liu, X., Roberts, J., Dakic, A., Zhang, Y., Schlegel, R., 2008. HPV E7 contributes to the telomerase activity of immortalized and tumorigenic cells and augments E6-induced hTERT promoter function. *Virology* 375, 611–623. <https://doi.org/10.1016/j.virol.2008.02.025>.
- McKinney, C.C., Hussmann, K.L., McBride, A.A., 2015. The role of the DNA damage response throughout the papillomavirus life cycle. *Viruses* 7, 2450–2469. <https://doi.org/10.3390/v7052450>.

- Mo, M., Shahar, S., Fleming, S.B., Mercer, A.A., 2012. How viruses affect the cell cycle through manipulation of the APC/C. *Trends Microbiol.* 20, 440–448. <https://doi.org/10.1016/j.tim.2012.05.007>.
- Venuti, A., Paolini, F., Nasir, L., Corteggio, A., Roperto, S., Campo, M.S., Borzacchiello, G., 2011. Papillomavirus E5: the smallest oncoprotein with many functions. *Mol. Cancer* 10, 140. <https://doi.org/10.1186/1476-4598-10-140>.
- Vinokurova, S., Wentzzenen, N., Kraus, I., Klaes, R., Driesch, C., Melsheimer, P., Kisseljov, F., Dürst, M., Schneider, A., von Knebel Doeberitz, M., 2008. Type-dependent integration frequency of human papillomavirus genomes in cervical lesions. *Cancer Res.* 68, 307–313. <https://doi.org/10.1158/0008-5472.CAN-07-2754>.
- Wang, J.W., Roden, R.B.S., 2013. L2, the minor capsid protein of papillomavirus. *Virology* 445, 175–186. <https://doi.org/10.1016/j.virol.2013.04.017>.
- Wang, Q., Griffin, H., Southern, S., Jackson, D., Martin, A., McIntosh, P., Davy, C., Masterson, P.J., Walker, P.A., Laskey, P., Omary, M.B., Doorbar, J., 2004. Functional analysis of the human papillomavirus type 16 E1=E4 protein provides a mechanism for in vivo and in vitro keratin filament reorganization. *J. Virol.* 78, 821–833.
- Zhou, Q., Zhu, K., Cheng, H., 2011. Ubiquitination in host immune response to human papillomavirus infection. *Arch. Dermatol. Res.* 303, 217–230. <https://doi.org/10.1007/s00403-011-1141-0>.

# Diseases of the blood



## OUTLINE

Anemia of chronic disease (inflammatory anemia)

97

Hemophilia

112

Blood disorders may affect one or more types of blood cells or plasma proteins. Plasma, the liquid part of the blood, is made of water, salts, and proteins. Blood cells perform different functions—erythrocytes (red blood cells) transport oxygen through the body, leukocytes (white blood cells) defend against infection, and platelets help control bleeding.

Decreased numbers of red blood cells and hemoglobin can cause anemia with symptoms such as fatigue, weakness, and shortness of breath. Decreased numbers of white blood cells can cause recurrent fever and infections. Decreased platelets or, separately, blood clotting factors can cause abnormal bleeding and bruising.

Blood cells develop in the bone marrow. The spleen, liver, thymus, and lymph nodes are also necessary for the normal function of blood cells and the synthesis of plasma proteins.

The most common diseases of the blood are anemia, disorders of platelets and the coagulation system, lymphoproliferative disorders, and myelodysplastic-myeloproliferative disorders.

In this chapter, we give two examples of blood disorders: anemia of chronic disease and hemophilia. Anemia in general involves a decrease in the number of red blood cells or a decrease in hemoglobin levels, which in turn decrease the oxygen-carrying capacity of the blood. Anemia of chronic disease or the anemia of inflammation is the most common cause of anemia in patients admitted to the hospital. Anemia of chronic disease can be a sign of a variety of conditions including chronic diseases such as cancer, certain infections, and a number of autoimmune diseases.

Disorders of platelets and the coagulation system cause bleeding or, conversely, excessive blood clotting (termed hypercoagulation) and thrombosis. Hemophilia or inherited genetic coagulopathy results in the decreased ability of the blood to form clots and stop the bleeding. Replacement therapy with blood clotting factors is currently the only available treatment for hemophilia, although gene therapy is hoped to be a solution in the future.

In this chapter, we do not provide examples of the molecular pathogenesis of blood cancers. However, lymphoproliferative and myelodysplastic-myeloproliferative disorders that can lead to cancer are truly common blood disorders. Lymphoproliferative disorders include both chronic and acute lymphocytic leukemia, and they are characterized by the high rate of proliferation of lymphocytes into a monoclonal lymphocytosis. Myelodysplastic-myeloproliferative disorders are characterized by abnormalities of myeloid blood cell differentiation and clonal expansions of blood cells. They include both chronic and acute myeloid leukemia and a number of other conditions.

## CHAPTER

## 3.1

### Anemia of chronic disease (inflammatory anemia)

Anemia of chronic inflammation is the preferred name for this disease since not all chronic illnesses are related to this form of anemia.

AI/ACD is often found in chronic infection, chronic immune activation, and some malignancies (Weiss and Goodnough, 2005).

Inflammatory anemia or anemia of chronic disease (AI/ACD) is a disorder of iron homeostasis promoted by hepcidin in response to an inflammatory condition. (Ferri and Ferri, 2018).

Clinical presentations of AI/ACD include palpitations, headache, generalized weakness, and dizziness. The severity of these symptoms may vary widely depending on the severity of the accompanying anemia (Ferri and Ferri, 2018).

Iron is required for hemoglobin (Hb) synthesis and for oxygen transport from the lungs to the rest of the body. Hb is synthesized in the bone marrow throughout the early differentiation stages of erythrocyte precursors—from pronormoblast to reticulocyte. When iron is lacking in the human body (as in iron-deficiency anemia) or sequestered (as in AI/ACD), erythropoiesis is impaired, and the number of erythrocytes decreases. This results in the diminished capacity of the blood to carry oxygen (Goodnough, 2012). The protein hepcidin (HAMP) that is released into the circulation by liver hepatocytes plays an essential role in AI/ACD progression. HAMP normally inhibits iron transport by binding to the iron transporter (SLC40A1) located on both gut enterocytes and reticuloendothelial cells (macrophages).

In AI/ACD, inflammatory mediators (such as the interleukins) stimulate HAMP production and its release from the liver.

**Pathway 1.** Inflammatory mediators increase the production of hepcidin (HAMP) (Fig. 1).

High levels of HAMP lead to the inhibition of the channel for iron export (SLC40A1), which in turn impairs intestinal iron absorption.

**Pathway 2.** High levels of hepcidin block iron absorption in the gut (Fig. 2).

HAMP also impairs iron release from splenic macrophages through the same mechanism.

**Pathway 3.** *Impairment of iron release from splenic macrophages (Fig. 3).*

HAMP-independent mechanisms of AI/ACD development are related to less effective erythropoiesis under inflammatory conditions. The GATA transcription factors can be downregulated, or erythropoietin production can be impaired.

**Pathway 4.** *Inflammatory mediators inhibit GATA signaling in erythropoiesis (Fig. 4).*

**Pathway 5.** *The rationale for the treatment of anemia of chronic disease with erythropoietin (Fig. 5).*

## Key cellular contributors and processes

Erythropoiesis

Process

Erythropoiesis is the process of red blood cell formation that occurs in the bone marrow.

Proinflammatory cytokines

Proteins

Cytokines are a large number of small proteins released by immune cells that participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFNG), granulocyte-macrophage colony-stimulating factor (GMCS-F), and others) are secreted primarily by macrophages and T-helper cells to upregulate proinflammatory reactions.

## Pathway 1

### Inflammatory mediators raise the production of hepcidin ([Fig. 1](#))

#### Cause and inductors

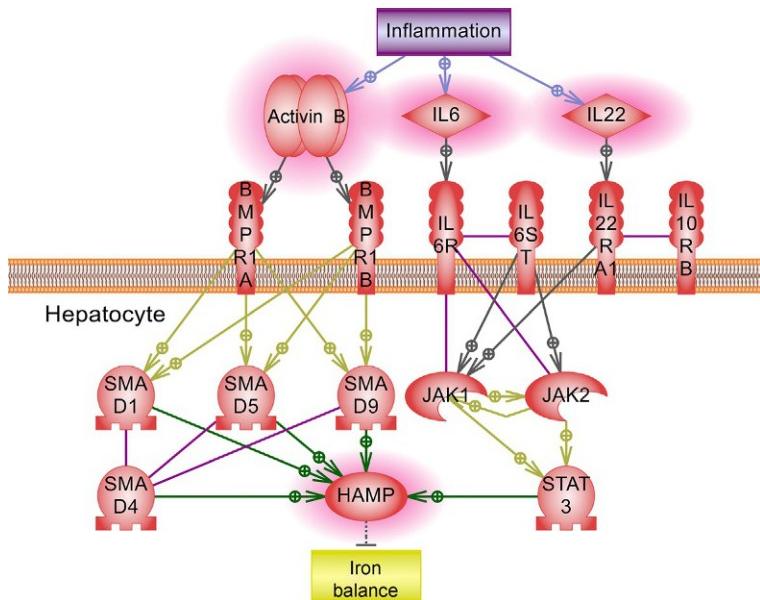
During general inflammation, there is an increase in the synthesis of interleukins and other mediators by different types of cells. The interleukins IL-6 and IL-22 and also activin B are thought to be the primary regulators of inflammatory anemia ([Fung and Nemeth, 2013](#); [Weiss and Goodnough, 2005](#)). Inflammatory mediators induce production of HAMP by hepatocytes.

#### Outcome effects

HAMP released to the blood from the liver regulates the systemic iron balance—it prevents iron release from enterocytes and macrophages (see [Pathways 2](#) and [3](#)). In concert with its evolutionary origin, hepcidin-related hypoferremia serves as a defense mechanism against iron-dependent pathogens, but in the case of chronic infection, this defense mechanism turns into a disease ([Ganz and Nemeth, 2012](#); [Hentze et al., 2010](#)).

#### Signaling

First, IL-6 and IL-22 both stimulate JAK/STAT3 signaling via their respective receptors IL-6R and IL-22RA1. Activin B positively regulates the transcription factors SMAD1/SMAD5/SMAD9/SMAD4 through the bone morphogenetic protein receptors (BMPR1A/B). Then the transcription factors STAT3 and SMADs bind to the HAMP gene promoter to stimulate transcription ([Fung and Nemeth, 2013](#)).



**FIG. 1** Pathway 1: Inflammatory mediators increase the production of hepcidin (HAMP).

## Pathway 2

### High levels of hepcidin block iron absorption in the gut (Fig. 2)

#### Cause and inducers

The two main molecular forms of dietary iron are nonorganic ( $\text{Fe}^{3+}$ ) and organic heme (including hemoglobin and myoglobin). Heme provides up to two-thirds of a person's daily iron intake in the developed world.

The low pH of the stomach helps release the heme-containing proteins hemoglobin and myoglobin from dietary meat. Most of the dietary iron is absorbed by enterocytes of the duodenum and the proximal jejunum. In order to be released into the blood after absorption, iron needs the transporter SLC40A1. HAMP can block the transport of iron by SLC40A1. HAMP is secreted from hepatocytes and circulates in plasma bound to alpha-2-macroglobulin (A2M). Interferon gamma, IFNG, may inhibit expression of SLC40A1 and therefore block iron absorption. The precise mechanism of this action is not clear.

#### Outcome effects

Iron is required for erythropoiesis, oxygen transport, and a plethora of other biological functions. When HAMP blocks the release of iron from enterocytes, anemia gradually develops.

#### Signaling

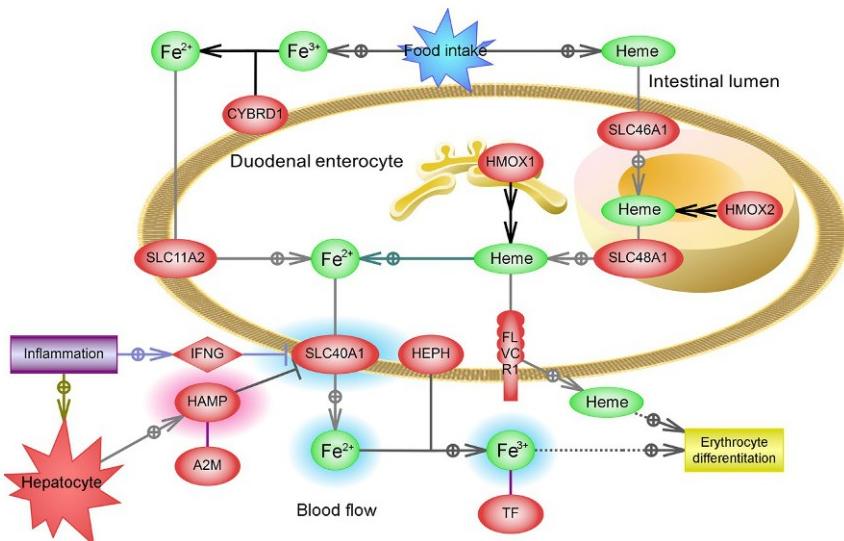
Inorganic dietary iron is absorbed by duodenal enterocytes (Hentze et al., 2010; Schmidt, 2015). At physiological pH, iron exists in the oxidized, trivalent ferric ( $\text{Fe}^{3+}$ ) state. To be absorbed, iron must be reduced to the divalent ferrous ( $\text{Fe}^{2+}$ ) state by the enzyme CYBRD1, which is located on the membrane of enterocytes (Hentze et al., 2010). Then, inorganic dietary iron is transported into cells by the divalent metal transporter SLC11A2 (DMT1).

Organic heme can be directly imported into enterocytes by HCP1 (SLC46A1) and by endocytosis. Heme can be exported from the endocytic vesicles to the cytoplasm by the heme transporter (HRG1, SLC48A1) and further metabolized by heme oxygenase 1 (HMOX1), which is present on the endoplasmic reticulum. This leads to the release of iron. At the same time, heme can be metabolized inside the endocytic vesicles by the enzyme HMOX2, which is localized on the vesicle membrane. In this case, iron needs to be exported to the cytoplasm.

Further the enterocytic cytosolic iron can be released into the blood-stream by means of the basolateral iron exporter SLC40A1 (Hentze et al., 2010; Schmidt, 2015). SLC40A1-dependent enterocytic iron export requires the enzyme hephaestin (HEPH). HEPH oxidizes Fe(2+) to Fe(3+), which is needed for iron to bind to transferrin (TF). When bound to TF, iron remains in a soluble form. TF is a major iron-carrier protein in mammals (Hentze et al., 2010).

A fraction of the intact heme can be released via the heme transporter FLVCR1 (Hooda et al., 2014).

Importantly, iron export from enterocytes can be inhibited by HAMP, which circulates in the blood bound to alpha-2-macroglobulin (A2M), and it can bind to and cause SLC40A1 degradation (Hentze et al., 2010).



**FIG. 2** Pathway 2: High levels of hepcidin block iron absorption in the gut.

## Pathway 3

### Impairment of iron release from splenic macrophages (Fig. 3)

#### Cause and inductors

Macrophages of the reticuloendothelial system play a major role in iron recycling. Less than 10% of daily iron needs come from intestinal absorption; the rest is provided by macrophages that recycle iron from senescent erythrocytes (Hentze et al., 2010; Schmidt, 2015).

In macrophage, inflammatory mediators upregulate the synthesis of the major intracellular iron storage protein ferritin and stimulate the ferritin-bound deposition of iron. In AI/ACD the levels of HAMP secreted from hepatocytes is higher than normal (Hentze et al., 2010; Schmidt, 2015). HAMP can inhibit iron export by binding to the iron transporter ferroportin (SLC40A1), which is present on macrophages to trigger its degradation. Bacterial infection may also cause the down-regulation of SLC40A1 synthesis (Weiss and Goodnough, 2005).

#### Outcome effects

Hypoferremia rapidly develops as a result of decreased macrophage iron release, which in turn causes anemia. Increases in the size of the ferritin-bound iron depository within macrophages may result in macrophage apoptosis and therefore promote inflammation.

#### Signaling

Free  $\text{Fe}^{2+}$  is imported into macrophages by the membrane transporter SLC11A2, whereas  $\text{Fe}^{2+}$  bound to TF is imported by the transferrin receptor (TFRC). Hemoglobin-derived heme is catabolized in macrophages by heme oxygenase (HMOX1); hence,  $\text{Fe}^{2+}$  is released into the cytosol. Proinflammatory cytokines such as IL-6, IL-10, IL-1B, and TNF upregulate ferritin expression and stimulate iron storage inside macrophages (Weiss and Goodnough, 2005).

Cytosolic iron is exported from macrophages by ferroportin (SLC40A1) and is coupled to oxidation by ceruloplasmin (CP), a protein synthesized and secreted by the liver (Hentze et al., 2010; Schmidt, 2015).

Interferon gamma (IFNG) and bacterial LPS inhibit SLC40A1 synthesis, thereby contributing to the impairment of iron balance in macrophages.

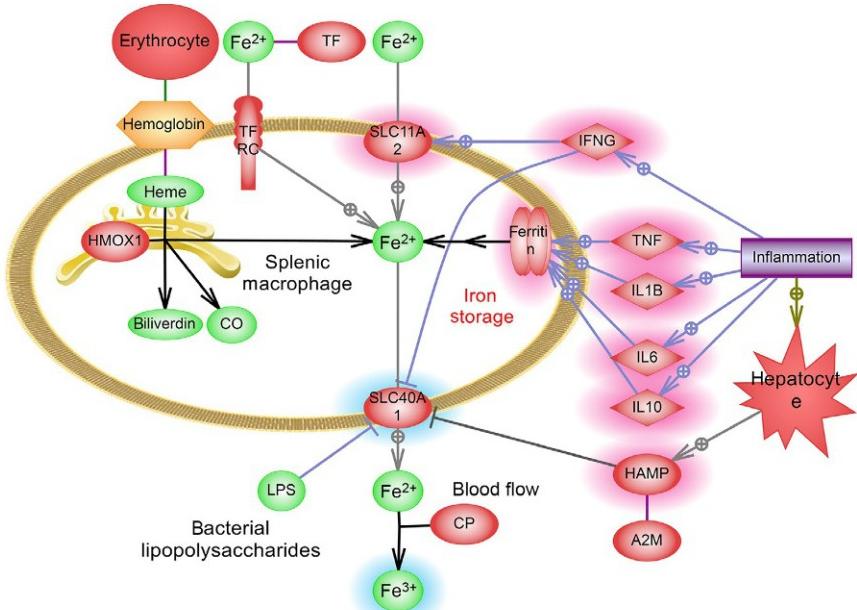


FIG. 3 Pathway 3: Impairment of iron release from splenic macrophages.

## Pathway 4

### Inflammatory mediators inhibit GATA signaling in erythropoiesis (Fig. 4)

#### Cause and inductors

Inflammatory mediators are directly involved in the regulation of erythrocyte differentiation. High levels of proinflammatory cytokines inhibit erythropoiesis by blocking the expression of the erythrocyte-specific proteins (HBA1, HBG1, AHSP, GYPA, and EPOR). The proinflammatory cytokines IL-6, TNF, and IFNG have a leading role in the inhibition of erythropoiesis (Morceau et al., 2009; Weiss and Goodnough, 2005).

#### Outcome effects

The low rate of expression and synthesis of erythrocyte-specific proteins slows down the differentiation of erythrocyte precursors.

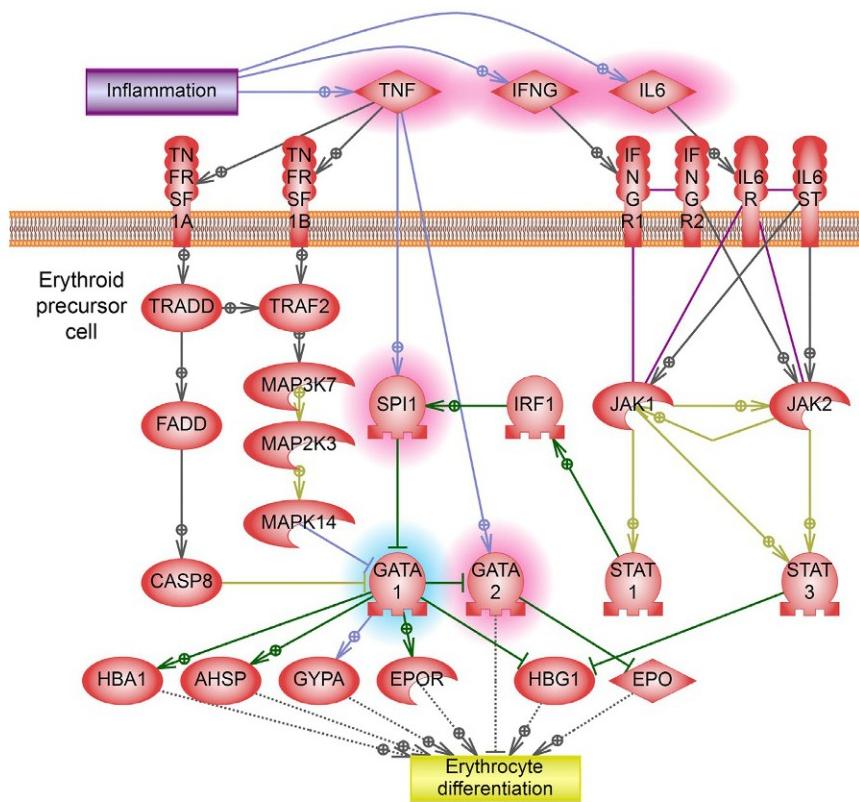
Lack of functional erythrocytes causes a systemic oxygen deficiency and the clinical manifestations of anemia.

#### Signaling

Tumor necrosis factor (TNF) acts through its receptor TNFRSF1A/B, which in turn activates MAPKs and inhibits the function of the erythroid-specific transcriptional factor GATA1. TNF and IFNG1 may inhibit GATA1 through the stimulation of another transcription factor, the protooncogene Spi1 (SPI1) (Libregts et al., 2011). GATA1 induces transcription of erythrocyte-specific proteins such as hemoglobin (HBA1) and the erythropoietin receptor (EPOR).

Also, TNF induces the expression of the transcription factor GATA2, which is itself a negative regulator of erythropoiesis (Bibikova et al., 2014; Grigorakaki et al., 2011).

IL-6 acts through its receptor IL-6R, and INF $\gamma$  works through the IFN $\gamma$ R1/IFN $\gamma$ R2 signaling cascades. Both IL-6 and INF $\gamma$  activate JAK1/JAK2-STAT3 transcription factor signaling, which in turn inhibits the expression of the hemoglobin subunit gamma 1 (HBG1).



**FIG. 4** Pathway 4: Inflammatory mediators inhibit GATA signaling in erythropoiesis.

## Pathway 5

### The rationale for anemia of chronic disease treatment with erythropoietin ([Fig. 5](#))

#### Cause and inductors

Endogenous and exogenous erythropoietins inhibit HAMP expression and stimulate erythrocyte differentiation. Erythropoietin (EPO) production is reduced in AI/ACD.

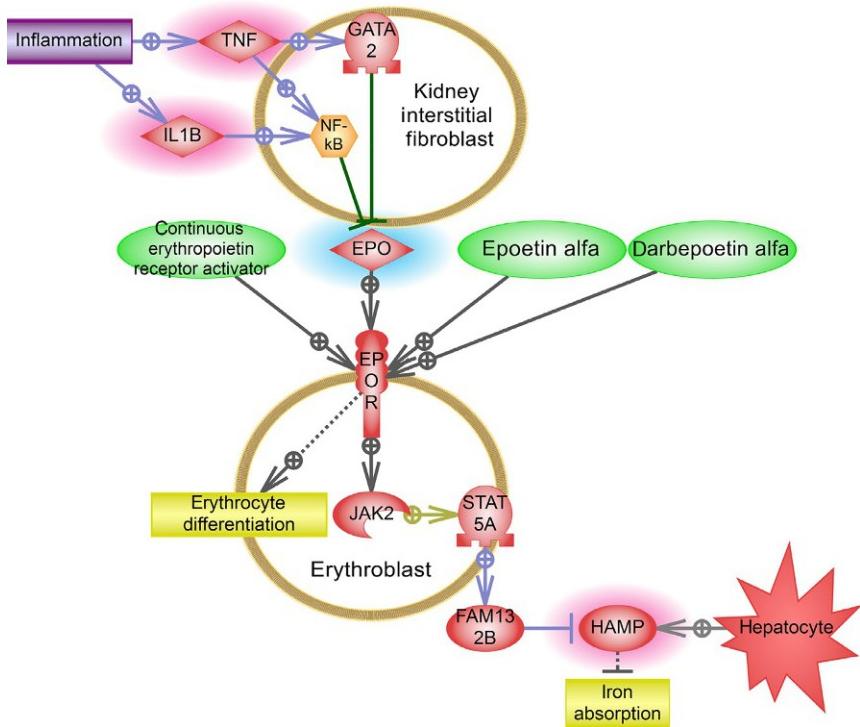
#### Outcome effects

Erythropoietin substitutive drugs are actively used for the treatment of AI/ACD. Restored EPO functions to reactivate erythrocyte differentiation and facilitate the inhibition of HAMP. The inhibition of HAMP returns iron exported from enterocytes, splenic macrophages, and hepatocytes to the blood and thereby restores the systemic iron balance.

#### Signaling

The expression of EPO in kidney fibroblasts is reduced due to the signaling of inflammatory mediators, such as TNF and IL-1B ([Weiss and Goodnough, 2005](#)). Mediators typically activate the GATA2 and NFKB transcription factors, which themselves inhibit *EPO* gene transcription ([Morceau et al., 2009](#)).

EPO and endogenous EPO analogs (e.g., epoetin alfa, darbepoetin alfa, and continuous erythropoietin receptor activator) activate the STAT5 transcription factor following the synthesis of erythrocferrone (FAM132B) in erythrocyte precursors. FAM132B, acting on hepatocytes, can reduce the expression of HAMP ([Schmidt, 2015](#)).



**FIG. 5** Pathway 5: The rationale for the treatment of anemia of chronic disease with erythropoietin.

## References

- Bibikova, E., Youn, M.-Y., Danilova, N., Ono-Uruga, Y., Konto-Ghiorghi, Y., Ochoa, R., Narla, A., Glader, B., Lin, S., Sakamoto, K.M., 2014. TNF-mediated inflammation represses GATA1 and activates p38 MAP kinase in RPS19-deficient hematopoietic progenitors. *Blood* 124, 3791–3798. <https://doi.org/10.1182/blood-2014-06-584656>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fung, E., Nemeth, E., 2013. Manipulation of the hepcidin pathway for therapeutic purposes. *Haematologica* 98, 1667–1676. <https://doi.org/10.3324/haematol.2013.084624>.
- Ganz, T., Nemeth, E., 2012. Hepcidin and iron homeostasis. *Biochim. Biophys. Acta* 1823, 1434–1443. <https://doi.org/10.1016/j.bbamcr.2012.01.014>.
- Goodnough, L.T., 2012. Iron deficiency syndromes and iron-restricted erythropoiesis (CME). *Transfusion (Paris)* 52, 1584–1592. <https://doi.org/10.1111/j.1537-2995.2011.03495.x>.
- Grigorakaki, C., Morceau, F., Chateauvieux, S., Dicato, M., Diederich, M., 2011. Tumor necrosis factor  $\alpha$ -mediated inhibition of erythropoiesis involves GATA-1/GATA-2 balance impairment and PU.1 over-expression. *Biochem. Pharmacol.* 82, 156–166. <https://doi.org/10.1016/j.bcp.2011.03.030>.
- Hentze, M.W., Muckenthaler, M.U., Galy, B., Camaschella, C., 2010. Two to tango: regulation of mammalian iron metabolism. *Cell* 142, 24–38. <https://doi.org/10.1016/j.cell.2010.06.028>.
- Hooda, J., Shah, A., Zhang, L., 2014. Heme, an essential nutrient from dietary proteins, critically impacts diverse physiological and pathological processes. *Nutrients* 6, 1080–1102. <https://doi.org/10.3390/nu6031080>.
- Libregts, S.F., Gutiérrez, L., de Bruin, A.M., Wensveen, F.M., Papadopoulos, P., van Ijcken, W., Ozgür, Z., Philipsen, S., Nolte, M.A., 2011. Chronic IFN- $\gamma$  production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. *Blood* 118, 2578–2588. <https://doi.org/10.1182/blood-2010-10-315218>.
- Morceau, F., Dicato, M., Diederich, M., 2009. Pro-inflammatory cytokine-mediated anemia: regarding molecular mechanisms of erythropoiesis. *Mediat. Inflamm.* 2009, 405016. <https://doi.org/10.1155/2009/405016>.
- Schmidt, P.J., 2015. Regulation of iron metabolism by hepcidin under conditions of inflammation. *J. Biol. Chem.* 290, 18975–18983. <https://doi.org/10.1074/jbc.R115.650150>.
- Weiss, G., Goodnough, L.T., 2005. Anemia of chronic disease. *N. Engl. J. Med.* 352, 1011–1023. <https://doi.org/10.1056/NEJMra041809>.

## CHAPTER

## 3.2

## Hemophilia

Hemophilia is a bleeding disorder that slows the blood clotting process after injury to blood vessels. The major types of this condition are hemophilia A (also known as classic hemophilia or factor VIII deficiency) and hemophilia B (also known as Christmas disease or factor IX deficiency).

Hemophilia is a hereditary bleeding disorder caused by low factor VIII coagulant activity (hemophilia A) or low levels of factor IX coagulant activity (hemophilia B). (*Ferri and Ferri, 2018*).

Hemophilia A and hemophilia B are genetic disorders caused by recessive mutations in single genes located on the X chromosome. Hemophilia A and B are caused by mutations in coagulation factor VIII (*F8*) and IX (*F9*), respectively. Different types of mutations may lead to hemophilia of varying severity. Milder forms of hemophilia may not become apparent until the affected individual undergoes surgery or experiences a serious injury. Continuous bleeding and even spontaneous bleeding happen in some cases of severe hemophilia, and serious complications can result from bleeding into internal organs.

Acquired hemophilia is a rare condition that usually begins in adulthood and is not caused by inherited genetic mutations. In acquired hemophilia, abnormal bleeding occurs in soft tissues. Acquired hemophilia may result from the production of autoantibodies against coagulation factor VIII (*F8*). The production of autoantibodies is sometimes associated with pregnancy, immune system disorders, cancer, or allergic reactions to certain drugs. However, in many cases, the etiology of acquired hemophilia remains unknown (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Hemophilia A and B are caused by mutations in coagulation factor VIII (*F8*) and IX (*F9*), respectively, which cause impairment of the coagulation cascade and the diminished capacity to form a blood clot, thereby predisposing affected individual to bleeding.

**Pathway 1. Dysfunction of coagulation cascade in hemophilia.**

Coagulation cascade (Fig. 6).

Feedback relations between coagulation and fibrinolysis (Fig. 7).

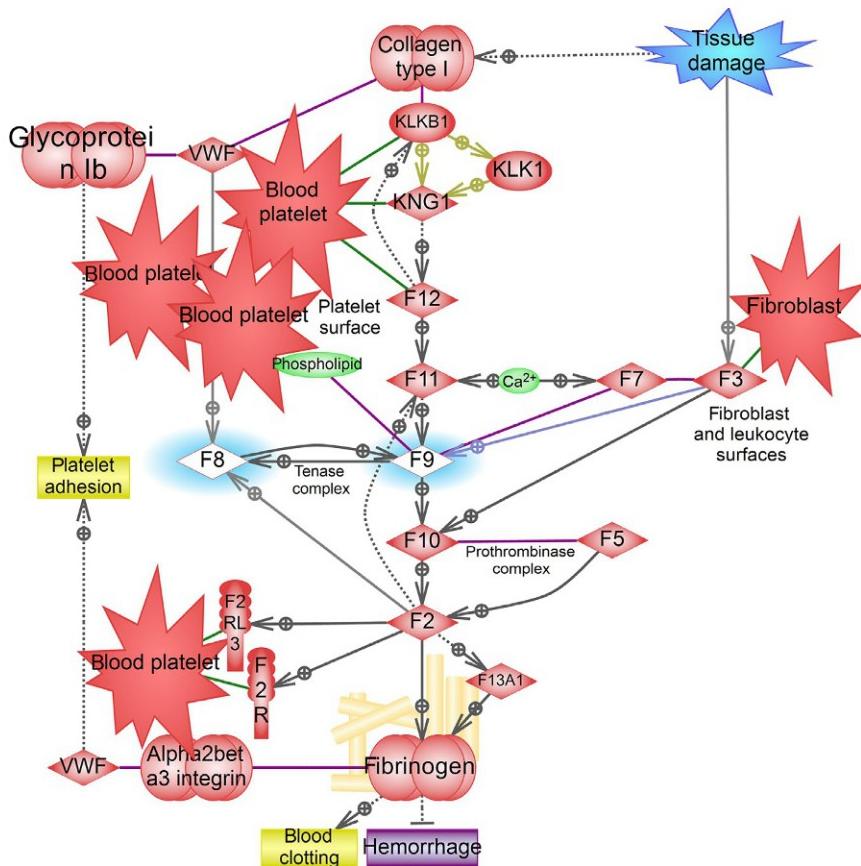


FIG. 6 Pathway 1: Dysfunction of coagulation cascade in hemophilia.

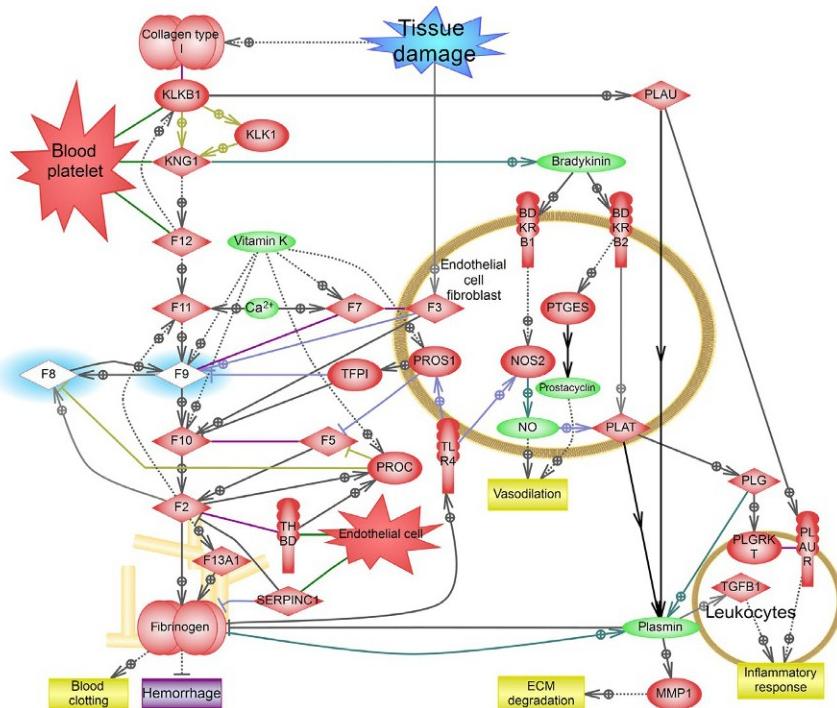


FIG. 7 Pathway 1: Feedback relations between coagulation and fibrinolysis.

## Key cellular contributors and processes

### Coagulation cascade

#### Process

The coagulation cascade is a complex set of reactions triggered in response to vascular damage that involve platelets and clotting factors, which together lead to the formation of the fibrin clot.

### Platelet

#### Cell

A platelet is a small circulating nonnucleated cellular fragment derived from megakaryocytes in the bone marrow. Platelets play a critical role in hemostasis (the process that stops bleeding from a ruptured vessel) and thrombosis (the formation of a clot within a blood vessel obstructing the blood flow).

## Pathway 1

### Dysfunction of the coagulation cascade in hemophilia

#### Cause and inductors

Hemophilia is caused by defective coagulation factors F8 and F9, which are normally involved in the coagulation cascade and blood clotting (Palta et al., 2014; Zimmerman and Valentino, 2013).

More than 900 genetic mutations are known to cause hemophilia A alone. There have been attempts to collect all known mutations in the *F8* gene to identify genotype/phenotype correlations. For example, in patients in the United Kingdom, the most common mutations are thought to involve an intron 22 inversion. This mutation accounts for 16.6% of all mutations and 38% of those mutations causing severe disease (Green et al., 2008).

Missense mutations are the most prevalent mutations in the *F9* gene in patients with hemophilia (deletions), which often occur *de novo* (Giannelli et al., 1997).

Acquired hemophilia is caused by the development of self-recognizing autoantibodies directed against coagulation factors, most commonly against F8 (Kessler and Knöbl, 2015).

#### Outcome effects

When the genes encoding *F8* or *F9* are mutated, the coagulation cascade cannot efficiently convert fibrinogen to fibrin to form an effective blood clot, thereby provoking recurrent bleeding. Treatment regimens that replace the missing blood clotting factors are the solution that works for most patients. Successful clinical trials of gene therapy approaches using recombinant adeno-associated viral vectors have been reported recently (Doshi and Arruda, 2018).

#### Signaling

##### **Coagulation cascade (Fig. 6)**

During the coagulation cascade, blood is transformed from a liquid to the gel form, and a clotting plug composed of fibrinogen and other molecules is formed. Coagulation usually begins after vascular injury when platelet adhesion and fibrin formation “cover” the damaged area to stop the bleeding (Palta et al., 2014; Zimmerman and Valentino, 2013).

The coagulation cascade is historically divided into three pathways although this division is artificial. These are the contact activation pathway (also known as the intrinsic pathway) and the tissue factor

pathway (also known as the extrinsic pathway), which both activate the “final common pathway” involving factor X (F10) (Palta et al., 2014; Zimmerman and Valentino, 2013). The majority of proteins involved in the coagulation cascade are produced by cells of blood vessels or the liver and can circulate as precursors of proteolytic enzymes. Some coagulation factors need  $\text{Ca}^{2+}$ , phospholipids, and vitamin K/VKORC1 for activation (Palta et al., 2014).

During the contact activation pathway, a complex of proteins that includes coagulation factor XII (F12), kallikrein B1 (KLKB1), and kininogen 1 (KNG1) are gathered on collagen molecules near the platelet surface. During activation, protein F12 is cleaved into two chains, and the light chain is referred to as activated F12. The activated F12 remains in close contact with the platelet surface and activates F11, which in turn activates F9. The subsequent step in the intrinsic pathway requires F8,  $\text{Ca}^{2+}$ , and membrane phospholipids to proceed efficiently to F10 activation. Another protein, known as von Willebrand factor (VWF), acts as a chaperone protein for F8 (Palta et al., 2014; Zimmerman and Valentino, 2013).

The extrinsic pathway leads to a much faster activation of F10 after tissue injury. Following damage to the blood vessel, coagulation factor VIII (F8) leaves the circulation and comes into contact with tissue factor (F3) expressed on stromal fibroblasts and leukocytes. The F3/F8 complex then rapidly activates F10.

The activation of F10 is ultimately responsible for the production of thrombin (F2), which converts fibrinogen to fibrin. F10 and F5 form the prothrombinase complex, which activates the conversion of prothrombin to thrombin. The polymerized fibrin is stabilized by coagulation factor XIII (F13A1).

In addition to the fibrin network, the coagulation cascade induces platelet aggregation. F2 can induce platelet activation and adhesion through its receptors (F2R and F2RL3) on the platelet’s surface. Von Willebrand factor (VWF) acts as a bridge between endothelial collagen I and the platelet surface receptor glycoprotein Ib or the integrins, thus promoting adhesions among platelets (Palta et al., 2014).

The fibrin network and aggregated platelets stabilize the clot and form a plug that prevents blood loss (Palta et al., 2014; Zimmerman and Valentino, 2013).

### ***Feedback relations between coagulation and fibrinolysis (Fig. 7)***

There are many feedback loops between coagulation factors and their regulators, which are important to maintaining the dynamic balance between thrombosis and fibrinolysis. An imbalance between regulators of the coagulation cascade may contribute to sporadic bleeding in patients with hemophilia.

Only major examples of such feedback relations between the activity of proteins and cells are shown in Fig. 7.

For example, thrombin (F2) has multiple effects on the coagulation cascade. F2 enhances the activation of F5 and F8 in a feedback manner, and it can also stimulate platelet activation and induce vasodilation through the production of the neurotransmitter NO (Motley et al., 2007).

Major anticoagulants include protein C (inactivator of coagulation factors Va and VIIIa, PROC), antithrombin (serpin, SERPINC1), and tissue factor pathway inhibitor (TFPI).

PROC is activated by the binding of F2 to thrombomodulin (THBD) on the endothelial cell surface. Activated PROC in turn degrades F5 and F8. Protein S (PROS1) also degrades F5 and F8.

TFPI blocks F10 very shortly after its activation by the F3–F7 complex. TFPI is expressed in the fibroblasts of subendothelial layer that contact the blood only in the event of vessel structural damage.

Vitamin K is the most potent cofactor for proteins of the coagulation system. A vitamin K deficiency contributes to hemorrhage.

The coagulation cascade interacts with other pathways such as the bradykinin and plasminogen activation systems. Plasminogen (PLG) is an inactive precursor of plasmin that is released from the liver. Upon binding to the cell surface, PLG is converted into active plasmin by PLAT, KNG1, F12, and other proteins from the coagulation cascade. Even fibrin is a cofactor for PLG activation (by PLAT). In turn, plasmin has fibrinolytic activity and an ability to degrade a variety of extracellular matrix proteins, but it is also involved with inflammation. Bradykinin causes blood vessel dilation to lower blood pressure. The biological actions of bradykinin are mediated by the B1 (BDKRB1) and B2 (BDKRB2) receptors that also mediate inflammation.

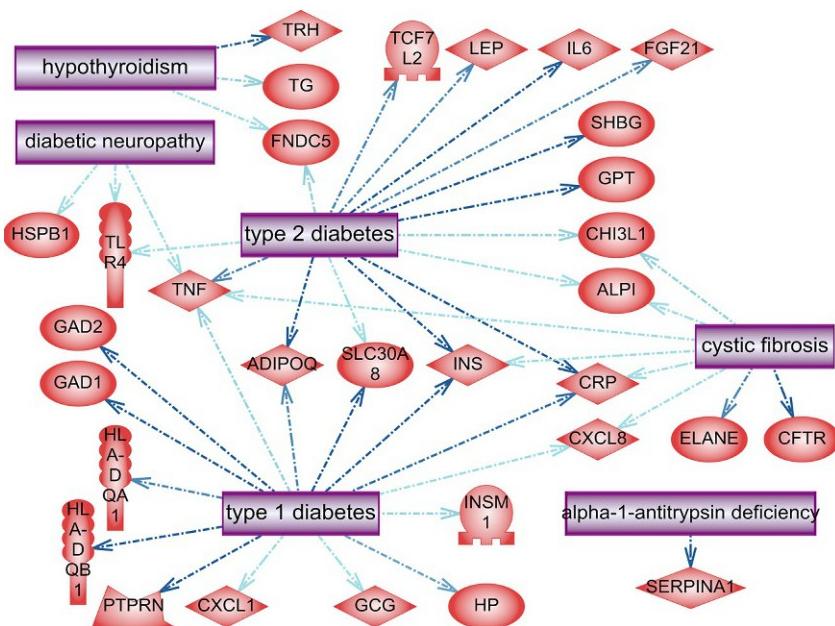
## References

- Disease number # 306700 and # 306900 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- Doshi, B.S., Arruda, V.R., 2018. Gene therapy for hemophilia: what does the future hold? *Ther. Adv. Hematol.* 9, 273–293. <https://doi.org/10.1177/2040620718791933>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Giannelli, F., Green, P.M., Sommer, S.S., Poon, M.C., Ludwig, M., Schwaab, R., Reitsma, P.H., Goossens, M., Yoshioka, A., Figueiredo, M.S., Brownlee, G.G., 1997. Haemophilia B: database of point mutations and short additions and deletions, 7th edition. *Nucleic Acids Res.* 25, 133–135.
- Green, P.M., Bagnall, R.D., Waseem, N.H., Giannelli, F., 2008. Haemophilia A mutations in the UK: results of screening one-third of the population. *Br. J. Haematol.* 143, 115–128. <https://doi.org/10.1111/j.1365-2141.2008.07310.x>.
- Kessler, C.M., Knöbl, P., 2015. Acquired haemophilia: an overview for clinical practice. *Eur. J. Haematol.* 95 (Suppl. 81), 36–44. <https://doi.org/10.1111/ejh.12689>.

- Motley, E.D., Eguchi, K., Patterson, M.M., Palmer, P.D., Suzuki, H., Eguchi, S., 2007. Mechanism of endothelial nitric oxide synthase phosphorylation and activation by thrombin. *Hypertension* 49, 577–583. <https://doi.org/10.1161/01.HYP.0000255954.80025.34>.
- Palta, S., Saroa, R., Palta, A., 2014. Overview of the coagulation system. *Indian J. Anaesth.* 58, 515–523. <https://doi.org/10.4103/0019-5049.144643>.
- Zimmerman, B., Valentino, L.A., 2013. Hemophilia: in review. *Pediatr. Rev.* 34, 289–294; quiz 295. <https://doi.org/10.1542/pir.34-7-289>.

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# Endocrine, nutritional, and metabolic diseases



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Endocrine, nutritional, and metabolic diseases are a heterogeneous group that includes “classical” endocrine diseases such as diabetes and hypothyroidism; nutritional diseases such as obesity; and metabolic disorders of proteins, fats, or carbohydrates such as alpha-1 antitrypsin deficiency and cystic fibrosis.

The endocrine system as a network of glands (including the thyroid gland, pituitary gland, adrenal gland, pancreas and others) and cells that produce and release hormones to control growth and development, reproduction, metabolism, mood, and other bodily functions. Characteristically, hormones travel through the blood to target tissues to stimulate different cellular processes in those distant tissues. The hyposecretion or hypersecretion of hormones and other molecules with signaling function may cause endocrine disorders. For example, the lack of the hormone insulin or insulin resistance causes diabetes; the lack of the triiodothyronine (T3) hormone causes hypothyroidism. Both of these are common diseases in modern society that will be described in this chapter.

The causes of endocrine, nutritional, and metabolic diseases can be very different. Lifestyle and environmental conditions inevitably influence the severity of these diseases and their recovery rate. However, heredity also plays an important role.

This chapter includes two metabolic disorders with mutations in single genes that not only alter lung function but also have more wider systemic effects. Alpha-1 antitrypsin deficiency is a genetic metabolic disorder that results in reduced function of alpha-1 antitrypsin protein, which in turn protects the body from the activity of destructive neutrophils. This disorder affects the lungs and liver. Cystic fibrosis (CF) is a genetic metabolic disorder that primarily affects not only the lungs but also the pancreas, liver, kidneys, and intestines. It is caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

Functional CFTR is needed for the influx of chloride ions and for the production of mucus, digestive fluids, and sweat. Defects in the CFTR protein result in osmotic imbalances that lead to the production of thick, viscous mucus. Most of the damage in CF is due to blockage of the narrow passages of affected organs with thickened secretions.

## CHAPTER

## 4.1

## Diabetes mellitus type I

Diabetes mellitus is a disorder characterized by abnormally high blood sugar levels. Type 1 diabetes is an autoimmune disorder that results from the immune system attacking beta cells of the pancreas. As a result, they stop producing insulin. Insulin is the major hormone that controls the amount of glucose taken up by cells from blood (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Diabetes mellitus (type I) refers to a syndrome of hyperglycemia resulting from many different causes results from autoimmune beta-cell destruction, usually leading to absolute insulin deficiency. (*Ferri and Ferri, 2018*).

Type 1 diabetes mellitus (DM, T1D, insulin-dependent diabetes mellitus (IDDM), or juvenile-onset diabetes) may start at any age, but onset is usually before 30 years. The American Diabetes Association (ADA) defines a person to have DM when symptoms of hyperglycemia are noticed, fasting plasma glucose (FPG) is  $\geq 126$  mg/dL, and certain indicators of an oral glucose tolerance test (OGTT) are checked (*Ferri and Ferri, 2018*).

Most T1D patients present the signs of autoimmune reactions, such as circulating antibodies against islet cell autoantigens, antiinsulin, anti-GAD65, antiinsulinoma antigen 2, or anti-ZNT8 (*Burbelo et al., 2012*). Untreated autoimmune destruction of the beta cells leads to a lifelong irreversible insulin deficiency. The lack of insulin and the related decrease in cellular glucose levels can lead to severe complications such as diabetic ketoacidosis (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

In T1D, the autoimmune reaction is initiated by a combination of hereditary and environmental factors. From the genetic point of view, T1D is a vastly heterogeneous illness caused by the effects of interplay between unrelated mutations in numerous genes. As of this writing, more than 50 genomic regions and more than 100 single nucleotide variants (SNVs) have been linked to T1D (<http://immunobase.org/disease/T1D/>) (*Wallet et al., 2017*). Typically, each patient has a unique combination of mutations associated with T1D risk; this greatly complicates the analysis and understanding of this disease and its triggers.

Slightly less than half of the patients with an increased risk for T1D have variants in the human leukocyte antigen (HLA) complex located on chromosome 6. This complex encodes proteins for the major histocompatibility complex (MHC). At the same time, some HLA variants appear to be protective against the disease ([Wallet et al., 2017](#)).

Moreover, a large number of variations in non-HLA genes also contribute to disease risk ([Ram and Morahan, 2017](#)). The status of the associative link between DM type 1 and mutations depends on the given population and a number of other factors. Variations in the PTPN22, INSULIN-IGF2, IL2RA, IFIH1, CLEC16A, and CTLA4 genes are examples that are considered high risk genotypes for DM type 1 ([Parkkola et al., 2017; Stankov et al., 2013](#)).

Mutated genes and the related dysfunction of immune system, environmental stress, and an unhealthy lifestyle exhaust the pancreas and lead to apoptosis of pancreatic cells. Pathogens, viruses, and apoptotic signals from dying cells drive the acceleration of beta-cell apoptosis, and in addition, they stimulate pancreatic antigen-presenting cells first followed by T-cell activation:

**Pathway 1.** *Antigen-presenting cells promote islet dysfunction and immune system activation in T1D* ([Fig. 1](#)).

During the prediabetes phase, impaired peripheral self-tolerance (apoptosis of T cells) in the pancreatic lymph node switches on the cell-mediated immune response against pancreatic cells and provokes B cells to express antibeta-cell autoantibodies. Mutations in the HLA genes and mutations (epigenetic changes, or posttranslational modifications) in several proteins secreted by beta cells allow the binding of MHC with self-antigens and activate T-cell expansion.

**Pathway 2.** *Defective tolerance of autoreactive T cells in T1D* ([Fig. 2](#)).

In early diabetes, progressive beta-cell dysfunction and apoptosis induced by the action of autoantibodies and of cytotoxic T-cell attack cause metabolic abnormalities including the disruption of insulin secretion and glucose metabolism.

**Pathway 3.** *Islet beta-cell destruction in T1D* ([Fig. 3](#)).

## Key cellular contributors and processes

Antigen-presenting cells

Cell

Antigen-presenting cells (APCs) are a large group of various cell types that trigger the cellular immune response by processing an antigen and exposing it on the cell's surface in a form recognizable by T cells in the process known as antigen presentation.

Diabetic ketoacidosis

Disease

Diabetic ketoacidosis is a metabolic complication of diabetes characterized by hyperglycemia, decreased serum pH, and increased serum levels of ketones.

Hyperglycemia

Process

Hyperglycemia refers to an abnormally high blood sugar level, and it is a hallmark of diabetes.

Ischemia

Process

Ischemia is the restricted blood supply to a tissue or organ caused by an obstruction or narrowing of a blood vessel.

Prediabetes

Disease

Prediabetes is an intermediate condition with glycemic numbers above normal but below the range diagnostic of diabetes.

The human leukocyte antigen complex

Protein

The human leukocyte antigen (HLA) complex is the group of genes located on chromosome 6 that encode proteins of the major histocompatibility complex (MHC).

The major histocompatibility complex II

Protein

The major histocompatibility complex (MHC) class II is a heterodimeric protein complex expressed on the surface of antigen-presenting cells. MHC class II molecules have a fundamental role in processing extracellular antigens and presenting them to T cells.

### Tissue-resident T cells

#### Cell

Tissue-resident T cells are a subset of T cells that reside in tissues without recirculating in the blood. They provide enhanced protection against infections that enter through body surfaces.

#### NOD-like receptors

#### Protein

The nucleotide-binding oligomerization domain–like receptors (NOD-like receptors, or NLRs) are cytoplasmic pattern recognition receptors. NLRs can bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) inside the cell. They have a variety of functions in the regulation of inflammatory and apoptotic responses. The NLR family consists of several proteins that are classified into subfamilies based on their N-terminal protein-interacting domains.

## Pathway 1

### **Antigen-presenting cells promote islet dysfunction and immune system activation in T1D (Fig. 1)**

#### **Incoming signals**

Antigen-presenting cells such as dendritic cells or macrophages detect substances released upon beta-cell death, bacterial or viral molecules, and, in response, produce large amounts of inflammatory molecules and T-cell stimulators. Compounds from dying beta cells are taken up by conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs) in the pancreatic islets and are presented as self-antigens to islet-specific T cells in the pancreatic lymph nodes.

Then the pancreatic beta cells respond to proinflammatory signals by initiating apoptosis and expressing additional inflammatory and immune system mediators.

#### **Outcome effects**

cDCs and pDCs loaded with self-antigens activate the differentiation of islet-specific T cells and promote the expansion of cells of both the T helper type 1 and T helper type 2 lineages. Activation of the immune and the inflammatory responses recruit macrophages, neutrophils, and cytotoxic T cells to the islet area that then destroy beta cells and enhance the existing insulitis in the pancreas. Although the role of dendritic cells in the progression of diabetes is complex, it clearly serves both a pathological and protective role.

#### **Signaling**

Conventional dendritic cells (cDCs) and plasmacytoid DCs (pDCs) are professional cells that can detect specific molecules and parts of molecules including damage (or pathogen)-associated molecular patterns (DAMP and PAMP). The toll-like receptors TLR2 and TLR4 are the primary receptors involved with the detection of DAMP and PAMP molecules by dendritic cells. TLR7 and TLR9 detect viral RNA or DNA and in turn stimulate the production of high levels of type 1 interferons (IFN). Other toll-like receptors are expressed on the membranes of pancreatic beta cells for the detection of microbial products (not shown on the pathway for simplicity).

TLRs trigger cytokine expression by activating NF- $\kappa$ B nuclear transcription factor signaling in dendritic cells. Activated dendritic cells express a large number of cytokines, such as the interleukins 12 (IL12A and IL12B)

or chemokines like CCL2, to promote a cell-mediated immune response. The pathway only shows several secreted cytokines and other molecules.

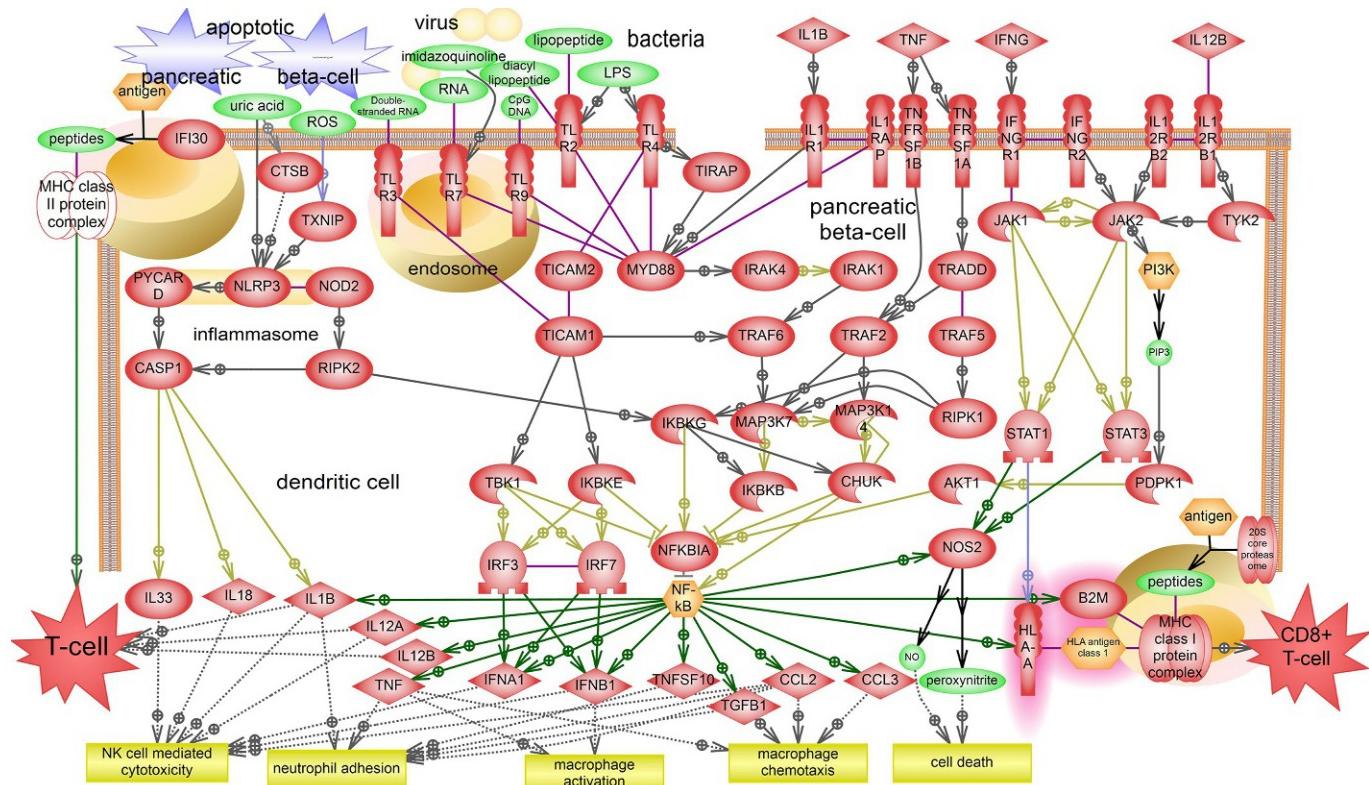
The inflammasome and NOD-like receptors are other critical intracellular DAMP sensors in dendritic cells. The inflammasome promotes the activation of the proinflammatory interleukins IL33, IL18, and IL1B.

Dendritic cells also absorb molecules that have been recognized as antigens by phagocytosis (the phagocytosis activation is not shown). Loaded with antigens, cDCs and pDCs from patients with early diagnosed DM type 1 migrate to pancreatic lymph nodes to present their antigens to islet-specific T cells with the help of the MHC II complex ([Diana et al., 2011](#)).

The MHC class I protein complex is responsible for the transmission of intracellular antigens, including beta cell-specific proteins. HLA-I is a part of the MHC class I protein complex that attracts and activates CD8+ cytotoxic T cells, which are thought to be the most abundant destructive T-cell population involved with T1D progression. HLA-I proteins have elevated expression levels in beta cells at various stages of T1D progression. The reasons for this are not well understood. However, IFNG secreted from activated immune cells is considered to be the driver of the observed HLA-I overexpression. The expression of beta-2-microglobulin (B2M), which is required for HLA class I complex stabilization, is also elevated in beta cells in some patients with T1D ([Richardson et al., 2016](#)).

Cytokines expressed from dendritic cells, such as IL1B, IL12B, IFNG, and TNF may cause direct damage (e.g., apoptosis) to beta cells. These ligands can trigger apoptosis through different mechanisms, for example, the activation of nitric oxide synthase 2 (NOS2) expression. NOS2 triggers nitric oxide (NO) and peroxynitrous acid related apoptosis ([Broniowska et al., 2014](#)).

## II. Human disease pathways



**FIG. 1** Pathway 1: Antigen-presenting cells promote islet dysfunction and immune system activation in T1D.

## Pathway 2

### Defective tolerance of autoreactive T cells in T1D (Fig. 2)

#### Inducing signals

Dendritic cells carrying autoantigens (fragments of beta cell-specific proteins) migrate to the pancreatic lymph nodes where they trigger the maturation of autoaggressive T cells. Naïve T cells differentiate into several T-cell types, including CD4+ (helper) T cells and CD8+ (cytotoxic, killer) T cells. Importantly, the autoreactive CD8+ T cells that directly destroy pancreatic beta cells are the prevalent T-cell population observed in T1D development.

T-helper cells (Th cells) differentiate into two major subtypes: Type 1 (Th1) and Type 2 (Th2). IL4 is a major Th2 cytokine, and it is necessary for Th2 lineage development, T cell-mediated B-cell maturation, auto-antibody synthesis, and humoral immunity. Th1 cells characteristically produce TNF-beta (LTA) and IFN-gamma (IFNG), which induce macrophages and other cells to promote a cell-mediated immune response.

The mechanisms by which autoreactive and unnecessary T cells are eliminated during their maturation phase (self-tolerance) can be ineffective in T1D ([Pugliese, 2017](#)). It is currently not well understood exactly how self-tolerance is disrupted in patients with T1D.

Mutations in HLA genes and mutations or tissue-specific epigenetic regulation of beta cell-specific genes (e.g., insulin) in the thymus early in life may cause autoreactive T cells to survive. Mutations in the HLA-DQA1 and HLA-DQB1 loci (coding the alpha and beta chains of the HLA-DQ heterodimer, a subunit of MHC class II) are associated with T1D ([Parkkola et al., 2017](#)). The HLA-DQ loci are highly variable so both alpha/beta protein chains in the human HLA-DQ heterodimer have isoforms that can be responsible for antigen-binding specificity.

Also, incorrect posttranslational modifications (PTM) of proteins in beta cells during inflammatory stress may result in specific neoepitopes for MHC. These neoepitopes may have good affinity for the mutated HLA part of MHC and may participate in the release of autoreactive T cells from a peripheral pancreatic lymph nodes. Binding of the INSULIN A chain and the GAD2 (GAD65) peptide with HLA-DRB1 are valuable examples of neoepitopes in T1D ([Pugliese, 2017](#)).

The regulatory T-cell (Treg) population that develops from CD4+ T cells may fail to suppress autoreactive T cells during the development of peripheral tolerance ([Tan et al., 2014](#)). Details of the T-cell tolerance insufficiency in T1D that is mediated by Treg-cell dysfunction are not well understood. Several polymorphisms in the genes controlling T-cell development (e.g., *PTPN22*, *CTLA4*, and *IL2RA*), have strong associations with T1D.

## Outcome effects

In diabetes mellitus type 1, impaired peripheral tolerance to autoantigens including insulin leads to the release of autoaggressive T cells (especially CD8 T lymphocytes) from the pancreatic lymph nodes.

There are numerous negative and positive feedback loops that regulate innate immune cells. For example, IFNG can further enhance its own production; IL12 produced by activated monocytes/macrophages and dendritic cells is the dominant factor for promoting Th1 cell differentiation; and IDO/kynurenine, IL10, and TNF-beta from DCs together promote Treg-cell function.

Upon viral infection of the pancreas, invariant natural killer T (iNKT) cells stimulate pDCs to express the antiviral IFN type 1. Cytokines from dendritic cells in turn activate iNKT cells and promote the production of anti-T-cell mediators (such as IL10) in the pancreatic lymph nodes. iNKT-pDC cross talk in the pancreas depends on TNFRSF4 ([Diana et al., 2011](#)).

## Signaling

There is no clear evidence to indicate how specific HLA variants relate to islet cell autoimmunity. According to accepted theory ([Sosinowski and Eisenbarth, 2013](#)), the interaction between HLA class II molecules, self-peptides (derived from insulin, for example), and T-cell receptors initiate the disease. Different variations of HLA-DQ molecules may bind to different islet autoantigen peptides. For example, antibodies to PTPRN (IA-2) and to INSULIN were shown to be correlated with HLA-DQ8 haplotype variants. GAD2 or SLC30A8 (ZnT8) autoantibodies associate with HLA-DQ2 ([Erlich et al., 2008; Maziarz et al., 2015](#)).

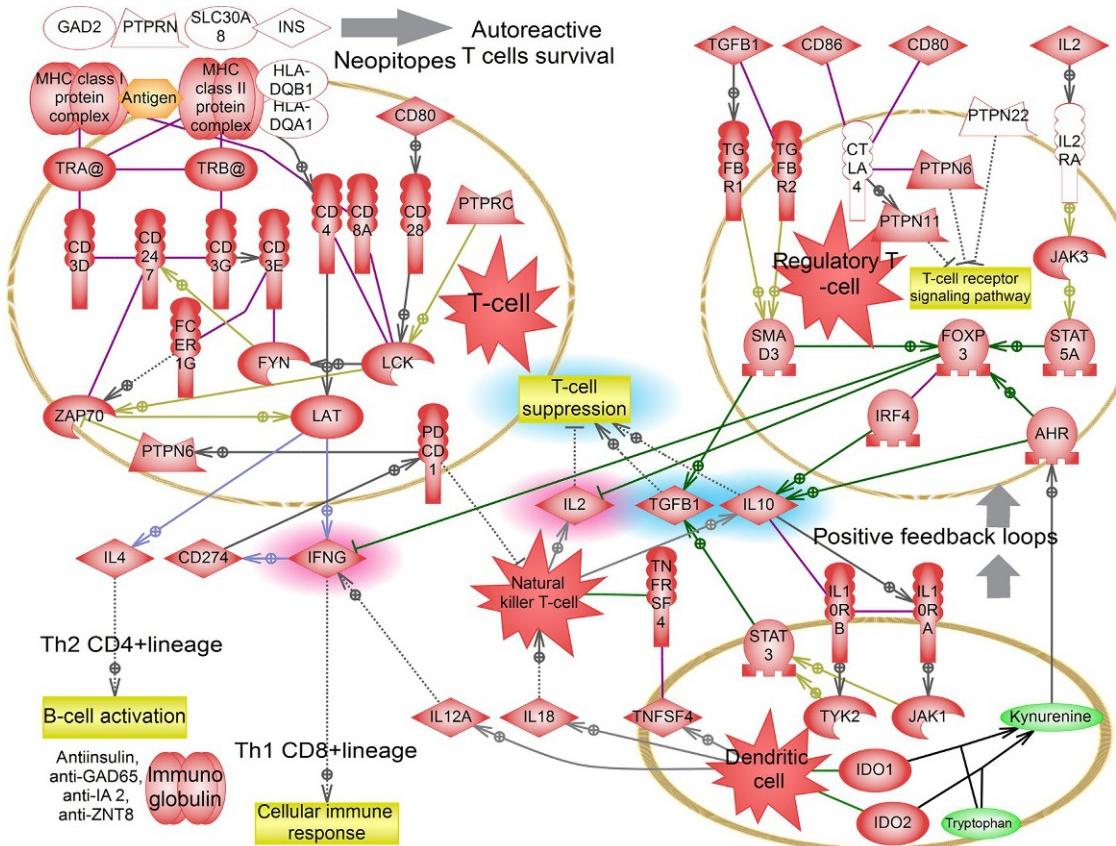
T-cell receptors (TCR) in naive T cells bind to antigen-MHC protein complexes on the surface of antigen-presenting cells. When CD4 or CD8 T cells bind to the antigen, they enter the next stage of differentiation that involves the positive selection of T cells. LCK-mediated phosphorylation of ZAP70 activates a variety of linker/adapter proteins such as LAT. Further signaling proteins are then recruited, which results in calcium mobilization, actin cytoskeleton reorganization, and activation of transcription factors such as NFAT and NF- $\kappa$ B. T-cell receptor signaling stimulates T-cell proliferation, differentiation, cytokine production, and other cellular responses (the complete signaling is not shown for the simplicity).

Peripheral tolerance occurs in the peripheral lymphoid organs (lymph nodes and spleen) following T-cell maturation. There are several critical mechanisms of peripheral T-cell tolerance, such as a lack of costimulation of TCR, robust TCR activation, and dephosphorylation of ZAP70 due to the arrangement of inhibitory receptors like PDCD1. In addition,

elevated amounts of proinflammatory ligands bind the death receptors FAS, TNFRSF25, and TNFRSF10A leading to the activation of apoptosis (not shown).

The normal role of Treg cells is to limit autoaggressive immune responses. Treg cells are characterized by the expression of CD4, IL2RA (CD25), CTLA4, FOXP3, and other markers. FOXP3 (forkhead box P3) is the main transcription factor that determines Treg differentiation and function. Antigen presentation by MHC II molecules and TCR activation are both necessary for FOXP3 activation in Treg cells. Also the survival of some types of Treg cells requires IL2/STAT5 and TGFB1 signaling in addition to FOXP3 expression. Treg cells may suppress T cells by releasing immunosuppressive cytokines such as interleukin 10 (IL10) and transforming growth factor beta (TGFB) or by other mechanisms.

## II. Human disease pathways



**FIG. 2** Pathway 2: Defective tolerance of autoreactive T cells in T1D.

## Pathway 3

### Islet beta-cell destruction in T1D (Fig. 3)

#### Inducing signals

Type 1 diabetes mellitus is an autoimmune pathology primarily driven by the action of autoreactive CD8+ cytotoxic T lymphocytes. Elevated expression levels of HLA-I proteins in beta cells in T1D may attract CD8+ T cells. CD8+ T cells can directly kill beta cells that express MCH class I (Richardson et al., 2016).

CD4+ T cells also are important in T1D development. They induce beta-cell apoptosis by stimulating cells of the innate immune system, by supporting the inflammatory response or by stimulating production of IFNG.

Other types of lymphocytes also take part in beta-cell destruction in T1D. Overall the disease pathogenesis involves entangled interactions and feedback loops between cells of the immune system. The pathway depicts only a few of them.

Furthermore, endoplasmic reticulum (ER) stress may also be an essential factor for promoting beta-cell dysfunction in T1D. Mutations in proteins secreted by beta cells or proteins of the ER folding machinery, lipotoxic stress, infection, and inflammation cause the accumulation of misfolded proteins in islet cells (Stankov et al., 2013).

#### Outcome effects

During the activation of the cell-mediated immune response, islet beta cells stop producing insulin and undergo apoptosis and pancreatic tissue becomes inflamed. Continued inflammation triggers further beta-cell dysfunction (Coppieters et al., 2012; Cunha et al., 2008; Fu et al., 2013; Kanatsuna et al., 2012; Pirot et al., 2008; Pugliese, 2017).

#### Signaling

Autoaggressive CD8+ T lymphocytes kill pancreatic beta cells. Recognition of MHC class I protein complexes carrying self-antigens on the surface of beta cells by T lymphocytes and the CD2-CD58 and ITGAL-ICAM1 interactions between cells result in CD8+ T-cell activation and the release of proteolytic granzymes (GZMA/GZMB), FASLG, and IFNG.

IFNG, FASLG, and GZMB promote apoptosis through the caspase cascade. IFNG binds to IFN $\gamma$ R1 on the beta-cell surface. The activated receptor in turn activates JAK1 and JAK2, which transduce the signal to the nuclear STAT1/IRF1 transcription factors. IRF1 binds to the *CASP1* promoter region and induces its expression. CASP1 cleaves CASP3 to initiate apoptosis.

The binding of FAS on the beta-cell membrane to FASLG on CD8+ T cells activates apoptotic signaling in the beta cell through the interaction of the cytoplasmic domain of FAS with the signaling adaptors FADD and DAXX. They in turn activate the caspase proteolytic cascade. CASP8 and CASP10 are activated first; then, they are cleaved and activate downstream caspases and several cellular substrates that lead to cell death.

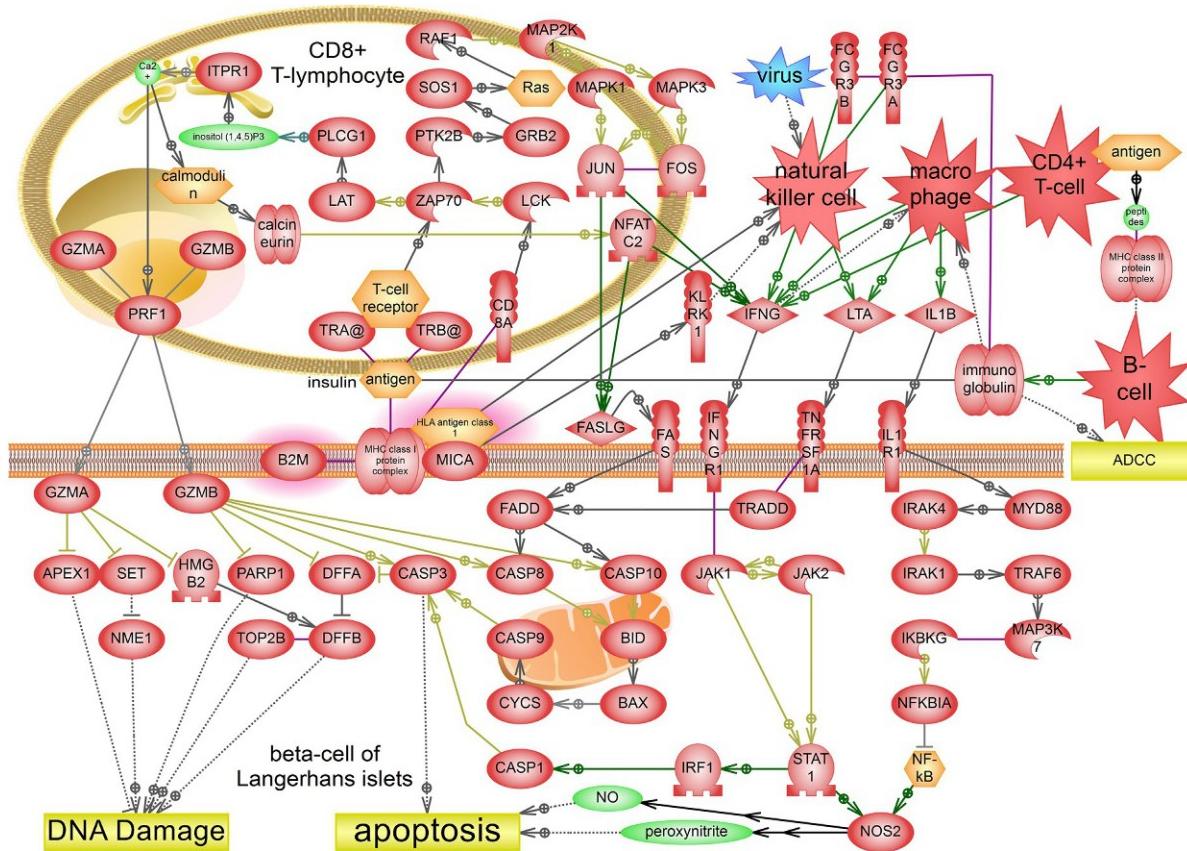
Also, there is a caspase-independent mechanism of GZMB-mediated apoptosis involving DNA fragmentation factor subunit beta (DFFB). Activated DFFB interacts with chromatin components such as topoisomerase II (TOP2B), which results in DNA fragmentation and apoptosis.

Macrophage-derived TNFB (LTA) and IL1B also contribute to beta-cell death through the caspase cascade. LTA binds to its receptor, TNFRSF1A, and stimulates further signal transduction leading to CASP10 and CASP2 activation. IL1B stimulates the induction of NOS2, nitric oxide (NO) generated by NOS2 initiates apoptosis.

Natural killer (NK) cells, such as CD8+ cytotoxic T cells, can be activated without binding to MHC I class complexes. NK-cell function is controlled by cytokines and interferons released from macrophages and dendritic cells (IL12, IL18, IL2, and others) during viral infection and by the binding of antibodies to FCGR3A/FCGR3B (CD16) receptors and the activation of killer-cell immunoglobulin-like receptors (such as NKG2D (KLRK1)). Activated NK cells release granzymes, IFNG, and TNFa to promote apoptosis in the target cell ([Diana et al., 2011](#)).

Finally, CD4+ T cells secrete cytokines that attract inflammatory cells and promote B-cell differentiation and antibody synthesis. Released antibodies then induce antibody-dependent cell-mediated cytotoxicity (ADCC) in the pancreas when different lymphocytes (eosinophils, macrophages, neutrophils, and NK cells) bind antibody-antigen complexes on the surface of beta cells to initiate apoptosis.

## II. Human disease pathways



## References

- Disease numbers #222100, #146880, #600716 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: E10. Endocrine, nutritional and metabolic diseases (E00-E90).
- Broniowska, K.A., Oleson, B.J., Corbett, J.A., 2014.  $\beta$ -cell responses to nitric oxide. *Vitam. Horm.* 95, 299–322. <https://doi.org/10.1016/B978-0-12-800174-5.00012-0>.
- Burbelo, P.D., Lebovitz, E.E., Bren, K.E., Bayat, A., Pavoli, S., Wenzlau, J.M., Barriga, K.J., Rewers, M., Harlan, D.M., Iadarola, M.J., 2012. Extrapancreatic autoantibody profiles in type I diabetes. *PLoS One* 7, e45216. <https://doi.org/10.1371/journal.pone.0045216>.
- Coppelters, K.T., Dotta, F., Amirian, N., Campbell, P.D., Kay, T.W.H., Atkinson, M.A., Roep, B.O., von Herrath, M.G., 2012. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J. Exp. Med.* 209, 51–60. <https://doi.org/10.1084/jem.20111187>.
- Cunha, D.A., Hekerman, P., Ladrière, L., Bazaar-Castro, A., Ortis, F., Wakeham, M.C., Moore, F., Rasschaert, J., Cardozo, A.K., Bellomo, E., Overbergh, L., Mathieu, C., Lupi, R., Hai, T., Herchuelz, A., Marchetti, P., Rutter, G.A., Eizirik, D.L., Cnop, M., 2008. Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *J. Cell Sci.* 121, 2308–2318. <https://doi.org/10.1242/jcs.026062>.
- Diana, J., Gahzarian, L., Simoni, Y., Lehuen, A., 2011. Innate immunity in type 1 diabetes. *Discov. Med.* 11, 513–520.
- Erlich, H., Valdes, A.M., Noble, J., Carlson, J.A., Varney, M., Concannon, P., Mychaleckyj, J.C., Todd, J.A., Bonella, P., Fear, A.L., Lavant, E., Louey, A., Moonsamy, P., for the Type 1 Diabetes Genetics Consortium, 2008. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes* 57, 1084–1092. <https://doi.org/10.2337/db07-1331>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fu, Z., Gilbert, E.R., Liu, D., 2013. Regulation of insulin synthesis and secretion and pancreatic beta-cell dysfunction in diabetes. *Curr. Diabetes Rev.* 9, 25–53.
- Kanatsuna, N., Papadopoulos, G.K., Moustakas, A.K., Lenmark, A., 2012. Etiopathogenesis of insulin autoimmunity. *Anat. Res. Int.* 2012, 457546. <https://doi.org/10.1155/2012/457546>.
- Maziarz, M., Hagopian, W., Palmer, J.P., Sanjeevi, C.B., Kockum, I., Breslow, N., Lernmark, Å., Swedish Childhood Diabetes Register, Diabetes Incidence in Sweden Study Group, Type 1 Diabetes Genetics Consortium, 2015. Non-HLA type 1 diabetes genes modulate disease risk together with HLA-DQ and islet autoantibodies. *Genes Immun.* 16, 541–551. <https://doi.org/10.1038/gene.2015.43>.
- Parkkola, A., Laine, A.-P., Karhunen, M., Härkönen, T., Ryhänen, S.J., Ilonen, J., Knip, M., Finnish Pediatric Diabetes Register, 2017. HLA and non-HLA genes and familial predisposition to autoimmune diseases in families with a child affected by type 1 diabetes. *PLoS One* 12, e0188402. <https://doi.org/10.1371/journal.pone.0188402>.
- Pirot, P., Cardozo, A.K., Eizirik, D.L., 2008. Mediators and mechanisms of pancreatic beta-cell death in type 1 diabetes. *Arq. Bras. Endocrinol. Metabol.* 52, 156–165.
- Pugliese, A., 2017. Autoreactive T cells in type 1 diabetes. *J. Clin. Invest.* 127, 2881–2891. <https://doi.org/10.1172/JCI94549>.
- Ram, R., Morahan, G., 2017. Effects of type 1 diabetes risk alleles on immune cell gene expression. *Genes* 8. <https://doi.org/10.3390/genes8060167>.
- Richardson, S.J., Rodriguez-Calvo, T., Gerling, I.C., Mathews, C.E., Kaddis, J.S., Russell, M.A., Zeissler, M., Leete, P., Krogvold, L., Dahl-Jørgensen, K., von Herrath, M., Pugliese, A., Atkinson, M.A., Morgan, N.G., 2016. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia* 59, 2448–2458. <https://doi.org/10.1007/s00125-016-4067-4>.

- Sosinowski, T., Eisenbarth, G.S., 2013. Type 1 diabetes: primary antigen/peptide/registry/trimolecular complex. *Immunol. Res.* 55, 270–276. <https://doi.org/10.1007/s12026-012-8367-6>.
- Stankov, K., Benc, D., Draskovic, D., 2013. Genetic and epigenetic factors in etiology of diabetes mellitus type 1. *Pediatrics* 132, 1112–1122. <https://doi.org/10.1542/peds.2013-1652>.
- Tan, T., Xiang, Y., Chang, C., Zhou, Z., 2014. Alteration of regulatory T cells in type 1 diabetes mellitus: a comprehensive review. *Clin. Rev. Allergy Immunol.* 47, 234–243. <https://doi.org/10.1007/s12016-014-8440-0>.
- Wallet, M.A., Santostefano, K.E., Terada, N., Brusko, T.M., 2017. Isogenic cellular systems model the impact of genetic risk variants in the pathogenesis of type 1 diabetes. *Front. Endocrinol.* 8, 276. <https://doi.org/10.3389/fendo.2017.00276>.

## CHAPTER

## 4.2

## Type 2 diabetes mellitus

Type 2 diabetes mellitus (DM2, T2D, noninsulin-dependent) is a disorder characterized by abnormally high blood sugar levels. Unlike T1D, the pathogenesis of T2D typically involves a combination of the death of insulin-producing cells in the pancreas (beta cells) and an increase in insulin resistance.

Type 2 DM can have a long presymptomatic phase, leading to a 4- to 7-year delay in diagnosis. Over time, DM2 can cause chronic and life-threatening health problems including cardiovascular disease, strokes, diabetic retinopathy, kidney failure, and blindness. (*Ferri and Ferri, 2018*).

As insulin resistance develops, cells use insulin less efficiently, so more insulin is needed to keep blood sugar levels within the normal range. Over time as T2D progresses, the number of pancreatic beta cells decreases, and they produce less insulin. Early symptoms of T2D include frequent urination (polyuria), excessive thirst (polydipsia), fatigue, blurred vision, tingling or loss of feeling in the hands and feet (diabetic neuropathy), and weight loss.

Initiation of the pathology of DM2 is complex and has various triggers such as a high-fat diet, obesity, or a genetic predisposition to hypertension. Although type 2 diabetes does not have a clear pattern of inheritance, at least 150 provoking and protective DNA variations have been associated with an increased risk of developing type 2 diabetes. The incidence of diabetes is rapidly growing worldwide largely due to changing lifestyles (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Diabetes mellitus type 2 is a pathology that slowly develops over time. Each stage of T2D is characterized by abnormalities on the cellular and tissue levels. At the prediabetes stages, a significant number of pancreatic beta cells become dysfunctional although they still secrete sufficient levels of insulin.

**Pathway 1. Insulin synthesis and secretion.**

Insulin processing and storage (Fig. 4).

Insulin secretion (Fig. 5).

High glucose levels, the production of inflammatory cytokines, and probably some genetic predisposition promote beta-cell death in the initial stages of the disease. At the time of T2D diagnosis, patients may have lost ~50% of their pancreatic beta cells ([Fonseca, 2009](#)):

**Pathway 2.** *Hyperglycemia and hyperlipidemia trigger beta-cell apoptosis* ([Fig. 6](#)).

As beta-cell death progresses, the pancreas compensates for the loss of beta cells by increasing insulin production. The mass of beta cell decreases until the ability of the pancreas to compensate for their loss is exceeded ([Marrif and Al-Sunousi, 2016](#)):

**Pathway 3.** *Beta-cell proliferation and compensation.*

Proliferation ([Fig. 7](#)).

Neogenesis/dedifferentiation ([Fig. 8](#)).

Recovery ([Fig. 9](#)).

During the overt (clear/apparent) stages of diabetes, defects in insulin receptor signaling and impaired insulin sensitivity (insulin resistance) develop throughout the whole body. Most older adults have some insulin resistance, but lifestyle and weight gain make it worse, greatly increasing the likelihood of developing T2D.

**Pathway 4.** *Insulin resistance.*

Insulin signaling ([Fig. 10](#)).

Inflammation-related insulin resistance ([Fig. 11](#)).

FFA-related insulin resistance ([Fig. 12](#)).

Once diabetes is developed, beta-cell destruction and decreased insulin secretion together lead to a lifelong insulin deficiency. The insulin deficiency is so severe that people become dependent on exogenous insulin for survival. Some organ-specific complications might also arise in the final stages of the disease (see Diabetic neuropathy and cataract).

## Key cellular contributors and processes

### Amylin

#### Protein

Amylin is a pancreatic peptide hormone cosecreted with insulin by pancreatic beta cells in response to nutrient stimuli. Amylin promotes satiety and acts as a partner of insulin to lower the blood glucose levels.

### C-peptide

#### Protein

C-peptide is a 31-amino acid long peptide, a by-product of insulin production, which is used in clinical tests as a marker of insulin production.

### Endoplasmic reticulum stress response

#### Process

The endoplasmic reticulum unfolded protein response (UPR) is a highly conserved adaptive process in eukaryotes that is triggered by a buildup of unfolded and/or misfolded proteins in the lumen of the endoplasmic reticulum. The UPR leads to the restoration of normal cellular functioning or the elimination of severely damaged cells via apoptosis.

### Hyperglycemia

#### Process

Hyperglycemia refers to abnormally high blood sugar levels and is a hallmark of diabetes.

### Insulin

#### Protein

Insulin is an anabolic peptide hormone that regulates glucose homeostasis by allowing glucose to enter cells, and it regulates the conversion of glucose into the storage molecule glycogen. Glycogen is metabolized during states of glucose deficiency.

### Insulin resistance

#### Process

Insulin resistance leads to higher than normal concentrations of insulin in the blood (hyperinsulinemia) as pancreatic beta cells produce more insulin in response to hyperglycemia caused by the inability of the cells to respond to insulin.

**Islet alpha cells****Cell**

Islet alpha cells are one of the major cell types in pancreatic Langerhans islands. When blood glucose and insulin levels decrease, alpha cells produce and secrete glucagon that regulates glycemia by stimulating glycogenolysis and gluconeogenesis and by blocking glycolysis in the liver hepatocytes.

**Islet beta cells****Cell**

Islet beta cells are another major cell type in the pancreatic Langerhans islands. Their main function is to synthesize and secrete insulin in response to elevated blood glucose levels.

## Pathway 1

### Insulin synthesis

#### Causes and inducers

The endocrine section of the pancreas (islets of Langerhans) consists of insulin-secreting beta cells and four other endocrine cell types. Adult beta cells are heterogeneous and differ by their glucose-responsiveness, insulin promoter activity, and insulin protein levels.

The primary endocrine function of pancreatic beta cells is to produce and release insulin in response to increasing blood glucose levels. Additional beta-cell products include an amyloid polypeptide (IAPP or amylin) and C-peptide, a cleavage product derived from proinsulin.

The secretion of insulin from pancreatic beta cells is a complex process. At the first step, insulin is synthesized as preproinsulin on ribosomes of the rough endoplasmic reticulum. Preproinsulin is then cleaved to produce proinsulin, which is transported to the Golgi apparatus to be packaged into secretory granules near the cell membrane.

#### Outcome effects

Insulin as a peptide hormone has many functions. The activation of anabolism by allowing glucose entry into the cell with its further conversion to carbohydrates and fats and by stimulating the synthesis of total protein pool is insulin's vital purpose. C-peptide may also influence the anabolism. The measurement of circulating C-peptide in the blood helps to diagnose different diabetes-related statuses. Amylin promotes satiety and acts as a partner of insulin to lower the glucose levels. Proamylin is considered one of the reasons for the loss of beta cells due to islet amyloid aggregation (Pillay and Govender, 2013).

#### Signaling

In beta cells, intracellular glucose is broken down to ATP during the process of glycolysis (see [Pathway 2](#)). The metabolism of glucose results in a  $\text{Ca}^{2+}$  influx into beta cells (see [Pathway 2](#)). Free  $\text{Ca}^{2+}$  activates phospholipase C (PLC), and additional  $\text{Ca}^{2+}$  is released from storage in the endoplasmic reticulum. A rise in free cytosolic  $\text{Ca}^{2+}$  initiates insulin synthesis and release.

Besides glucose, various hormones and neurotransmitters mediate insulin synthesis and secretion in pancreatic beta cells through G protein-coupled receptor signaling and by generating cAMP and increasing  $\text{Ca}^{2+}$  levels. For example, the gastric inhibitory polypeptide (GIP) and glucagon (GCG), through their receptors GIPR and GLP1R,

potentiate as much as 70% of the meal-induced insulin secretion in pancreatic beta cells (Fujita and Haneda, 2011).

### **Insulin gene expression (Fig. 4)**

The regulation of preproinsulin gene expression is a complex but well-studied process with knotted feedback mechanisms between various transcription factors and other proteins. We put only a few of them on the pathway illustrated.

Glucose metabolism is the most important physiological event that stimulates insulin gene transcription and mRNA translation (Poitout et al., 2006). Pancreatic and duodenal homeobox 1 (PDX1) is the major transcription factor regulating insulin expression. Several signaling pathways, including MAPK14, PI3K, and PASK, have been implicated in PDX1 phosphorylation and its nucleocytoplasmic shuttling.

Numerous other transcription factors, including CREB1, MAFA, NFE2L2, and PAX6, are involved in the regulation of human pancreatic beta-cell function. In animal models, glucose and glucagon (GCG) have been shown to regulate the nuclear localization and the transactivation capacity of PDX1 and neuronal differentiation 1 (NEUROD1) via post-transcriptional modifications. Both phosphorylation via MAPK3/MAPK1 and acetylation via E1A binding protein p300 (EP300) modify NEUROD1 (Andrali et al., 2008).

Also the transcription factor forkhead box O1 (FOXO1) helps insulin inhibit its own gene expression via PI3K/AKT signaling.

### **Insulin processing and storage (Fig. 4)**

During insulin synthesis, preproinsulin (24-residue signal peptide) is initially made. Proinsulin is made from preproinsulin in the lumen of the rough endoplasmic reticulum (ER). Proinsulin matures into active insulin through the action of cellular endopeptidases such as proprotein convertase subtilisin/kexin types 1 and 2 (PCSK1 and PCSK2) and carboxypeptidase E (CPE). PCSK1 and PCSK2 are themselves synthesized as inactive pro-PCSK1 and pro-PCSK2 precursors, which are activated by the specific chaperone proteins, PCSK1N and SCG5, respectively. In isolated rat pancreatic islets, the biosynthesis of PCSK1 is specifically stimulated by glucose. In contrast, PCSK2 biosynthesis is not glucose-regulated.

Functional endopeptidases cleave proinsulin to release a fragment called the C-peptide (connecting peptide) and two other peptide chains, B- and A-, which together comprise the mature insulin.

Insulin, C-peptide, and IAPP are then stored in the secretory granules in the beta cell. Insulin is stored as a crystal chelated with Zn<sup>2+</sup> in large dense-core secretory granules known as beta-granules. Beta-granules are continually turning over. If a beta-granule does not undergo exocytosis, it is retired by intracellular degradation with a half-life of

3–5 days (Alarcón et al., 1993; Uchizono et al., 2007). IAPP is secreted together with insulin in a 20:1 M ratio.

### **Insulin secretion (*Fig. 5*)**

The release of insulin from the pancreas is not continuous, but instead, it oscillates with a period of approximately 3–6 min. Pulsatile insulin secretion from individual beta cells is driven by the oscillation of intracellular  $\text{Ca}^{2+}$  levels, cyclic pacing of cellular metabolism, and the intracellular ATP/ADP ratio. The exocytosis of insulin occurs in two phases. The first phase is rapidly triggered in response to increased blood glucose levels, and it is controlled by the low-affinity  $\text{Ca}^{2+}$ -sensing mechanism (Henquin, 2000). Specifically, PCLO may act as a low-affinity  $\text{Ca}^{2+}$  sensor. PCLO facilitates rapid  $\text{Ca}^{2+}$ -induced exocytosis by interacting with the RAPGEF3/RAPGEF4 proteins. SYT9 has also been identified as a high-affinity  $\text{Ca}^{2+}$  sensor in beta cells.

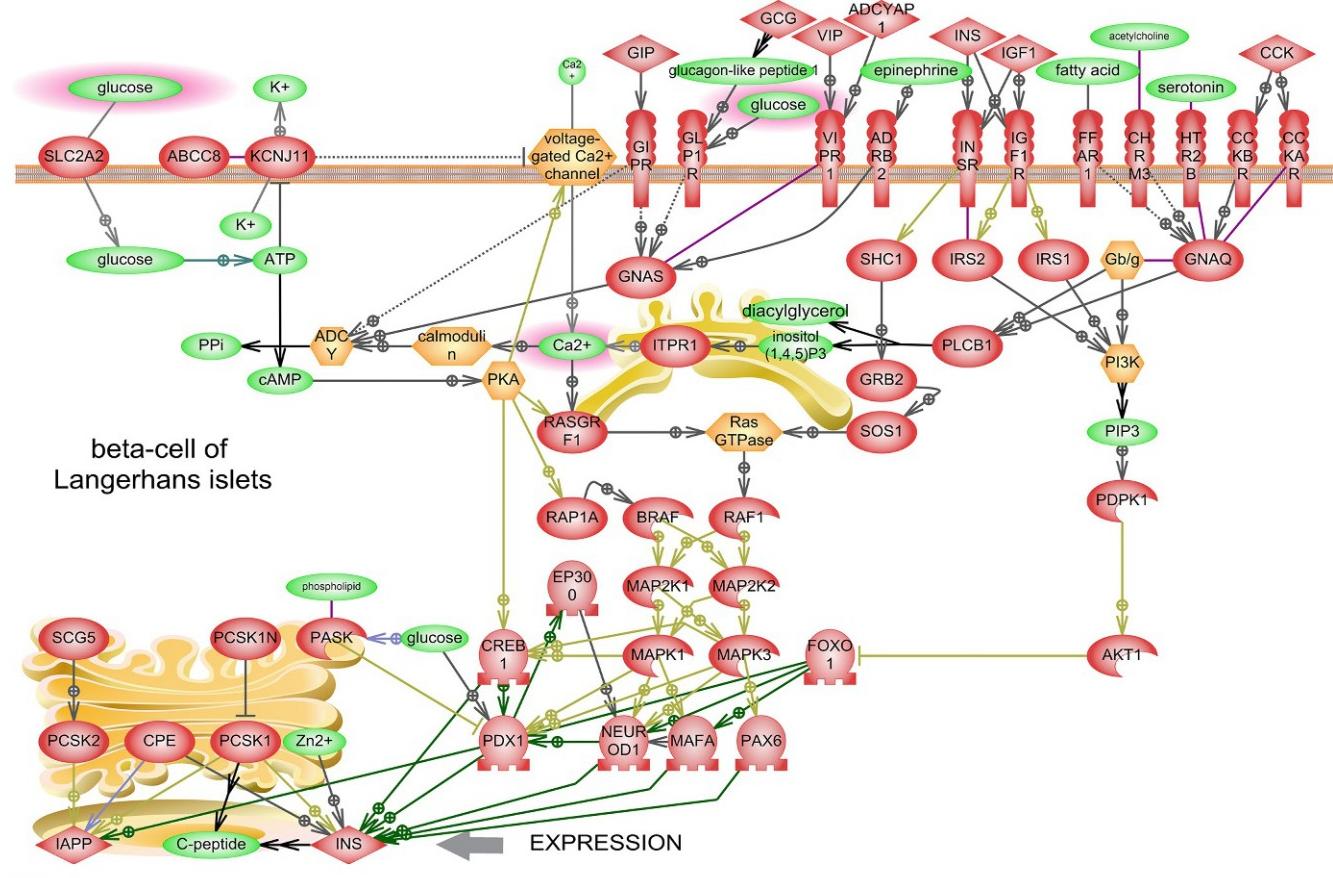
L-type CaV1.2 channels (CACNA1C) are responsible for the immediate release of insulin. R-type CaV2.3 channels (CACNA1E) are insufficient to drive insulin exocytosis, but they do accelerate granule mobilization (not shown). Only 1% of the granules with insulin (i.e., the readily releasable pool) are immediately available for the glucose-mediated secretion. The remaining granules belong to the reserve pool that has to undergo preparatory reactions before release (Ashcroft, 2005).

The process of insulin secretion, like any exocytosis event, involves secretory granule trafficking, tethering, docking, priming, and fusion with the cell membrane. Exocytosis is regulated by several protein complexes and cell signaling cascades. The fusion of granules involves complex interactions between SNAP25 (synaptosomal-associated protein) and STX1A (syntaxin 1A) at the plasma membrane, as well as the VAMP2 protein (vesicle-associated membrane protein 2 or synaptobrevin 2), and it is probably modulated by synaptotagmins (SYT9 and SYT7). Upon stimulation, proteolysis of SNAP25 seems to be critical for insulin secretion.

Cyclic AMP (cAMP) signaling promotes insulin exocytosis by facilitating the generation of  $\text{Ca}^{2+}$  signals by sensitizing the secretory machinery to  $\text{Ca}^{2+}$  and by stimulating the mobilization and priming of granules via the PKA- and RAPGEF4/RAPGEF3-dependent pathways. Notably, cAMP and  $\text{Ca}^{2+}$  are both influenced by intracellular ATP.

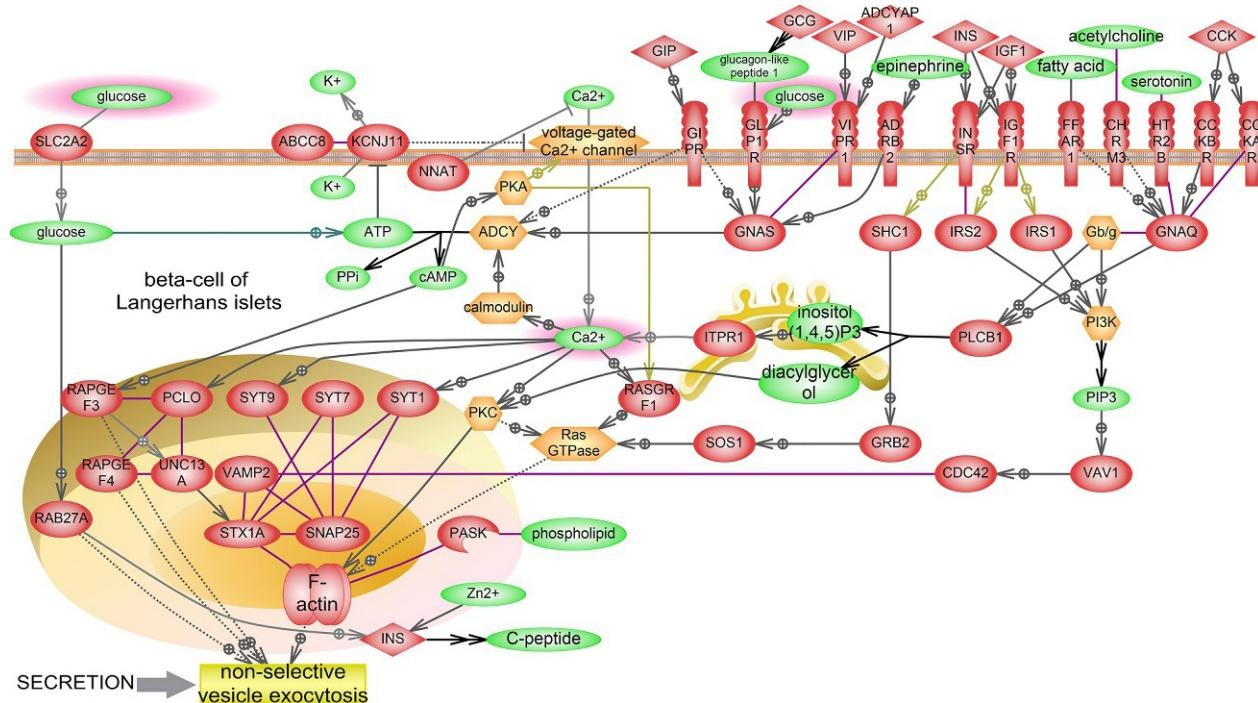
G protein-coupled receptor signaling regulates beta-cell  $\text{Ca}^{2+}$ -dependent exocytosis by controlling the size of secretory granules and the levels of F-actin agglomeration (Amisten et al., 2013).

Also, glucose may regulate insulin exocytosis in pancreatic beta cells by inducing the expression of exocytosis stimulating proteins such as SNAP25 and STX1A and the ion channels NNAT and ABCC8 (ATP-binding cassette transporter) (Shao et al., 2009).



**FIG. 4** Pathway 1: Insulin synthesis and secretion: insulin processing and storage.

## II. Human disease pathways



**FIG. 5** Pathway 1: Insulin synthesis and secretion: insulin secretion.

## Pathway 2

### Hyperglycemia and hyperlipidemia trigger beta-cell apoptosis (Fig. 6)

#### Causes and inductors

T2D disease progression is accompanied by endocrine pancreatic cell exhaustion resulting in beta-cell destruction. The destruction of pancreatic beta cells in type 2 diabetes mellitus occurs gradually, while excessive levels of intracellular glucose,  $\text{Ca}^{2+}$ , and long-chain saturated fatty acids (FFA) trigger cell apoptosis and increase oxidative stress. The primary source of oxidative stress in pancreatic beta cells most likely comes from reactive oxygen species (ROS) generated by the mitochondrial electron transport chain.

#### Outcome effects

ROS trigger oxidative stress in cells by directly injuring proteins, DNA, and the membranes of organelles. Activated  $\text{Ca}^{2+}$ -dependent caspases (CASP3, CASP9, and others) also cause cell damage. In addition, endoplasmic reticulum (ER) stress in beta cells triggers programmed cell death due to an accumulation of unprocessed proinsulin and other damaged proteins.

#### Signaling

Typically, glucose is transported into beta cells through facilitated diffusion with the help of the solute carrier family proteins SLC2A1 and SLC2A2 (GLUT1 and GLUT2) glucose transporters. Human beta cells predominantly express the glucose transporter SLC2A1 instead of SLC2A2, which is expressed in rodent beta cells.

Intracellular glucose is metabolized to ATP through glycolysis. A rise in the intracellular ATP/ADP ratio promotes ATP-sensitive potassium (K) channel (KCNJ11) closure, membrane depolarization, and the opening of voltage-gated calcium ( $\text{Ca}^{2+}$ ) channels. The opening of cell-surface voltage-dependent  $\text{Ca}^{2+}$  channels facilitates  $\text{Ca}^{2+}$  influx into beta cells. Therefore, an elevation in the ATP-to-ADP ratio induces cell membrane depolarization and the influx of  $\text{Ca}^{2+}$  into beta cells. An excessive amount of free  $\text{Ca}^{2+}$  triggers apoptosis induced by the mitochondria.

On the other hand, an excessive amount of glucose raises the level of b-nicotinamide-adenine-dinucleotide-phosphate (NADPH) derived from glycolysis. NADPH is used in the formation of nicotinamide adenine nucleotide hydride (NADH) in the tricarboxylic acid cycle, which

is a substrate for ATP production during mitochondrial oxidative phosphorylation. Importantly, intensive oxidative phosphorylation in mitochondria generates reactive oxygen species ( $H_2O_2$ , superoxide). CYP2E1 (cytochrome P450 2E) and the family of NADPH oxidases (NOX) could be another source of ROS.

Affected beta cells do not eliminate ROS due to their abnormally high levels. Moreover, levels of the ROS-inactivating enzymes, glutathione peroxidase (GPX1) and catalase (CAT), are low in human pancreatic beta cells and in cells from model animals. Human beta cells seem to be less prone to oxidative stress than rodent beta cells, possibly because they have higher CAT and superoxide dismutase 1 (SOD) activities. GPX1 activity is difficult to detect in human islets.

Chronic exposure to long-chain saturated fatty acids (FFA) is another risk factor for beta-cell apoptosis. The specific toxic effects of FFA may be related to ceramide formation and persistent endoplasmic reticulum (ER) stress. ER stress is caused by an accumulation of irreversibly misfolded or unfolded proteins that lead to beta-cell death. When FFA are esterified in the ER to form oleate and palmitate, they can induce the expression of ER stress-related proteins such as XBP1 and ATF6, or they may promote ER calcium depletion and fatty acid oxidation (not shown). Also, palmitate causes the rapid degradation of carboxypeptidase E (CPE), and this may contribute to ER stress (Cao et al., 2012; Shao et al., 2013) (Cnop et al., 2005; Johnson and Luciani, 2010).

## II. Human disease pathways

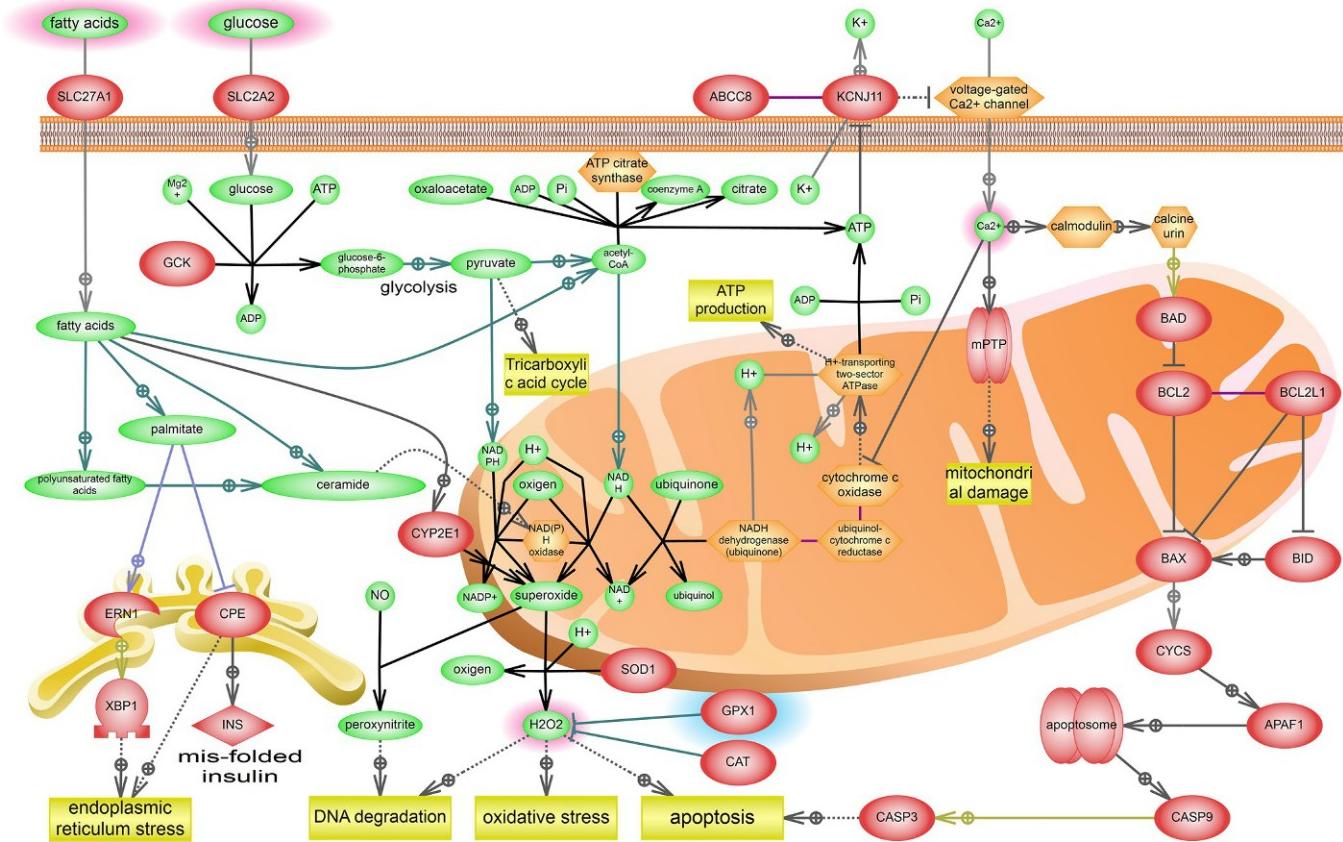


FIG. 6 Pathway 2: Hyperglycemia and hyperlipidemia trigger beta-cell apoptosis.

## Pathway 3

### Beta-cell proliferation and compensation

#### Causes and inductors

As diabetes progress, accompanying compensatory mechanisms induce increased insulin production by increasing the beta-cell population in the pancreas to regulate blood glucose levels. Beta-cell compensation stops when the ability of the pancreas to maintain and renew the beta-cell lineage is exhausted.

In humans the majority of beta-cell replication occurs only in early childhood. Studies have found no evidence of beta-cell replication in adults. The expansion of beta cells has been detected only during the neonatal period, pregnancy, and obesity.

#### Outcome effects

Details of the regulation of beta-cell renewal are still poorly understood. Instead of one single theory, various facts from different studies have been described here. Further, almost all facts about beta-cell proliferation or neogenesis originate from animal models and may not be entirely applicable to humans ([Kaiser and Leibowitz, 2009](#)). However, without any doubt, our understanding of the mechanisms of human beta-cell proliferation, recovery, and neogenesis is essential for finding methods to increase beta-cell numbers for the treatment of diabetes.

#### Signaling

##### **Proliferation (Fig. 7)**

Reasons for the renewal of beta-cell proliferation in obese adults can be multifarious. The effect could be modulated by changes in the synthesis of incretins (hormones that stimulate insulin secretion in response to meals such as GCG and GIP) and other hormones. It is believed that PI3K/AKT1 signaling is the central pathway involved in the regulation of beta-cell proliferation. Some factors such as insulin, the insulin-like growth factors IGF1/2, and incretin hormones increase beta-cell proliferation via enhanced IGF1R receptor activation and the related PI3K/AKT1 signaling in rat islets. During pregnancy (when the expansion of beta cells has also been detected), signaling of the PRL hormone triggers beta-cell proliferation through JAK2/STAT5A, thereby resulting in the direct activation of cyclin D2 (CCND2) expression and adaptive beta-cell hyperplasia.

Also, activation of the potent proliferative WNT signaling pathway has been shown to induce beta-cell proliferation. Mutations in an effector of WNT signaling, TCF7L2, have been linked to type 2 diabetes.

High blood glucose levels and the associated increase of intracellular calcium levels induce the proliferation of rodent beta cells by activating the calcineurin/NFATC1 signaling pathway. In human islet beta cells, calcineurin also has antiapoptotic effects.

However, high blood glucose levels along with the promotion of beta-cell proliferation cause glucolipotoxicity that contribute to the suppression of beta-cell proliferation through a feedback response. Glucolipotoxicity and proinflammatory cytokines activate EIF2AK2 (eukaryotic translation initiation factor 4E), which significantly inhibits rodent pancreatic beta-cell proliferation by arresting the cell cycle at G1.

Also, it was shown that in rat beta cells, dietary intake of carbohydrates and saturated fatty acids and treatment with the immunosuppressant medication “cyclosporine” may upregulate the sterol regulatory element binding transcription factor 1 (SREBF1), which in turn suppresses INS/IGF1/IRS2-related insulin secretion and beta-cell proliferation ([Alismail and Jin, 2014](#); [Sachdeva and Stoffers, 2009](#); [Soleimanpour et al., 2010](#)).

### ***Neogenesis/dedifferentiation (Fig. 8)***

Studies that directly measure beta-cell neogenesis have not been performed on humans. However, existing animal models may help clarify possible mechanisms of beta-cell neogenesis.

The pancreatic endoderm develops from the embryonic endoderm following the expression of PDX1. All islet endocrine cell types (endocrine fate) derive from a NEUROG3-positive cell in the pancreatic endoderm. Notch signaling represses NEUROG3 expression and maintains the cells' progenitor phenotype. Several days following the appearance of NEUROG3-positive cells, endocrine cells expressing insulin or glucagon (or both) appear in the cultures.

Following physiological stress and aging, mice beta cells lacking FOXO1 were hyperglycemic and had a reduced beta-cell mass. Lineage-tracing experiments in these FOXO1-deficient beta cells demonstrated that the loss of beta-cell mass was due to beta-cell dedifferentiation, not death. Dedifferentiated beta cells reverted to progenitor-like cells expressing NEUROG3, POU5F1, and NANOG. A subset of FOXO1-deficient beta cells adopted the alpha-cell fate, resulting in hyperglucagonemia. One hypothesis states that the INS/AKT1 pathway leads to the deactivation of FOXO1 and thus may decrease beta-cell proliferation mediated by PDX1. However, FOXO1 can still play a role in promoting the expression of INS. So, when the level of INS drops, active FOXO1 may contribute to the expansion of beta-cell mass but not to INS production.

Pancreas alpha cells can transdifferentiate into beta cells. Alpha cells produce glucagon and are similar to beta cells with regard to their intracellular signaling. The alpha-cell phenotype is determined by the expression of the ARX, PAX6, POU3F4, and other proteins and the absence of the PDX1, PAX4, and PCSK1/3 proteins and the GCG receptor. Fully-differentiated alpha cells express only PCSK2, and they produce glucagon. Also, MAFB is mostly absent from mature beta cells with its expression restricted to alpha cells in the adult pancreas. The signals that control alpha-cell proliferation and their conversion into beta cells remain unknown. Ectopic expression of PAX4 and the suppression of ARX are sufficient to convert alpha cells into beta cells *in vivo*. PAX6 regulates the expression of the MAF, MAFB, PCSK2, and NEUROD1 genes in addition to the glucagon gene itself (Collombat et al., 2009; Habener and Stanojevic, 2012; Szabat et al., 2012; Thorel et al., 2010).

### **Recovery (Fig. 9)**

In general, moderate elevations in glucose levels and increased expression of the incretins promote pancreatic beta-cell survival via the CREB1-mediated induction of insulin receptor substrate 2 (IRS2) expression (see Fig. 7, the proliferation of beta cell).

The stimulation of glucokinase (GCK) improves insulin secretion by the beta cells. PFKFB2 is a glucose sensor in glycolysis and an endogenous GCK activator. GCK activity is regulated by an adaptive translocation between the nucleus and the cytoplasm through the binding to and dissociation from its regulatory protein (GCKR).

The use of G6PC2 inhibitors may represent a new approach for the treatment of insulin secretion defects in type 2 diabetes. G6PC2 encodes an islet-specific glucose-6-phosphatase catalytic subunit located in the endoplasmic reticulum. Glucose-6-phosphatase catalyzes the dephosphorylation of glucose-6-phosphate to form glucose, a process that is the opposite of glucose utilization.

On the other hand, the inhibition of DPP4, the ubiquitous proteolytic enzyme for glucagon (GCG), improves islet and beta-cell functions by decreasing insulin synthesis/secretion levels. GCG is secreted from pancreatic alpha cells, and it stimulates glycolysis in beta cells under conditions of hypoglycemia and low levels of insulin.

Also, despite very controversial results, studies indicate that hunger may contribute to maintaining the healthy state of beta cells through action of the hormone ghrelin (GHRL, also called hunger hormone). Ghrelin is a gastric peptide hormone. It is one of the main hormones that stimulate hunger. In rat and human pancreatic tissues, the ghrelin receptor GHSR is expressed primarily in alpha cells and in some beta cells (Delporte, 2013). High concentrations of ghrelin may have an inhibitory effect on insulin secretion by activating ghrelin receptor (GHSR)/GNAI1 signaling. GHSR

is a G protein-coupled receptor that normally couples to GNAQ, which in turn leads to  $\text{Ca}^{2+}$  mobilization and the stimulation rather than the inhibition of insulin secretion. However, in rat islet and beta cells, GHSR couples to GNAI1 instead of GNAQ. GNAI1-related signaling reduces both cAMP accumulation and insulin secretion. GHSR coupling to GNAI1 rather than GNAQ requires interaction with the receptor SSTR5 and a high ratio of acylated ghrelin to somatostatin (SST). Ghrelin's inhibitory effects on insulin secretion are associated with enhanced expression of glycoproteins in the membranes of secretory granules, namely, PTPRN2. PTPRN2 is also the beta-cell autoantigen found in type 1 diabetes.

The mechanism for beta-cell recovery is related to the antioxidant effects of the transcription factor PPARG. Fatty acids or some drugs (such as the thiazolidinediones) activate the group of PPAR transcription factors with most profound specificity for PPARG. PPARG and NFE2L2 raise the activity of antioxidant enzymes in beta cells. The activation of PPARG also prevents NF- $\kappa$ B activation and the loss of calcium pump (ATP2A2) activity in the endoplasmic reticulum (Cullen et al., 2014; Delporte, 2013; Kaiser and Leibowitz, 2009; Park et al., 2012; Pound et al., 2013; Puddu et al., 2013).

## II. Human disease pathways

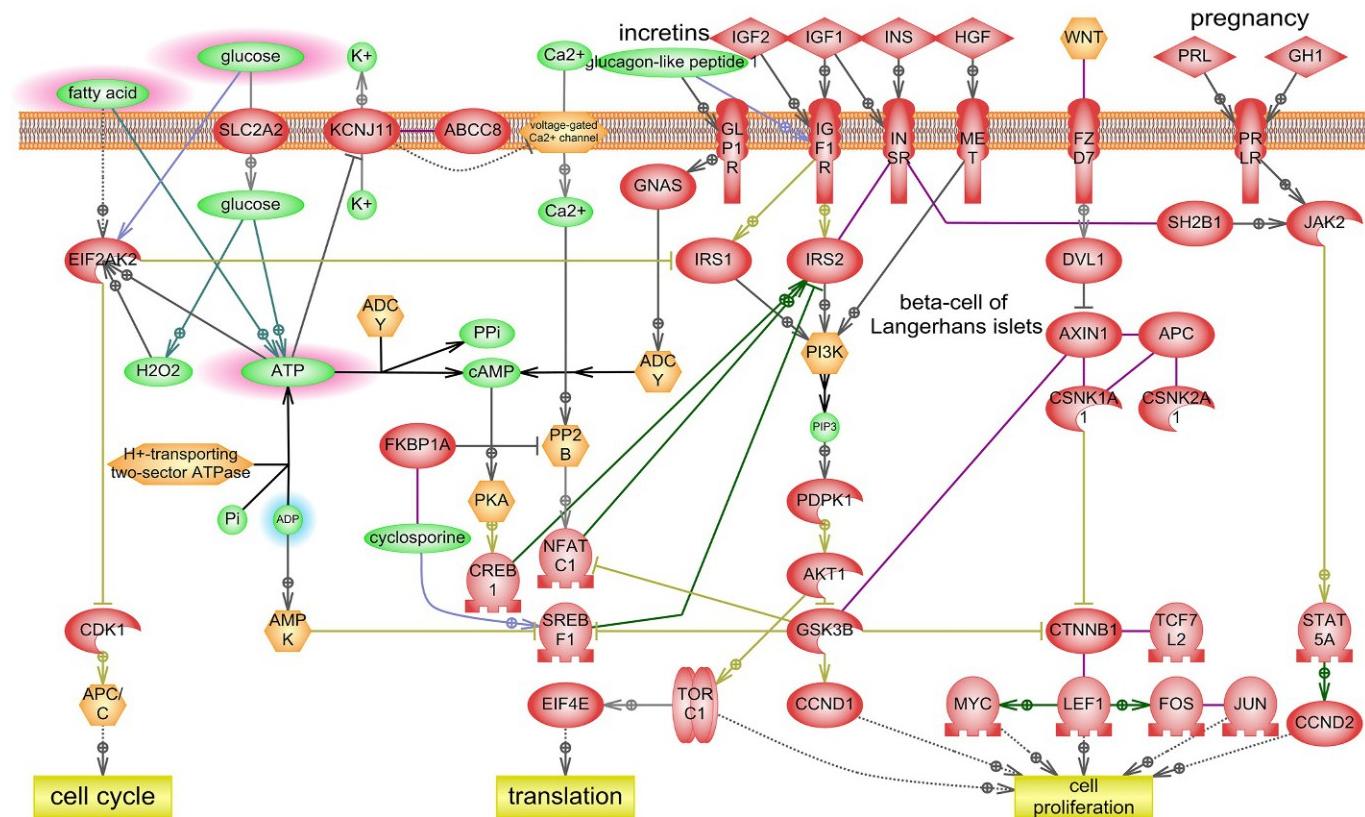
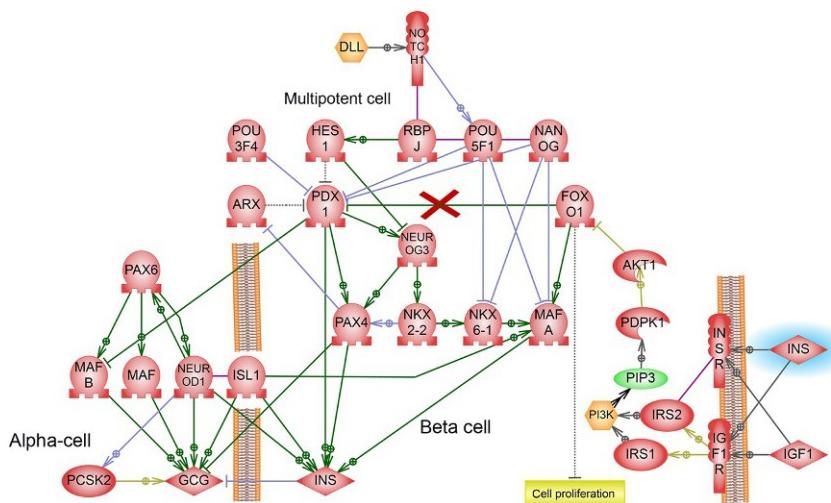


FIG. 7 Pathway 3: Beta-cell proliferation and compensation: Proliferation.



**FIG. 8** Pathway 3: Beta-cell proliferation and compensation: Neogenesis/dedifferentiation.

## II. Human disease pathways

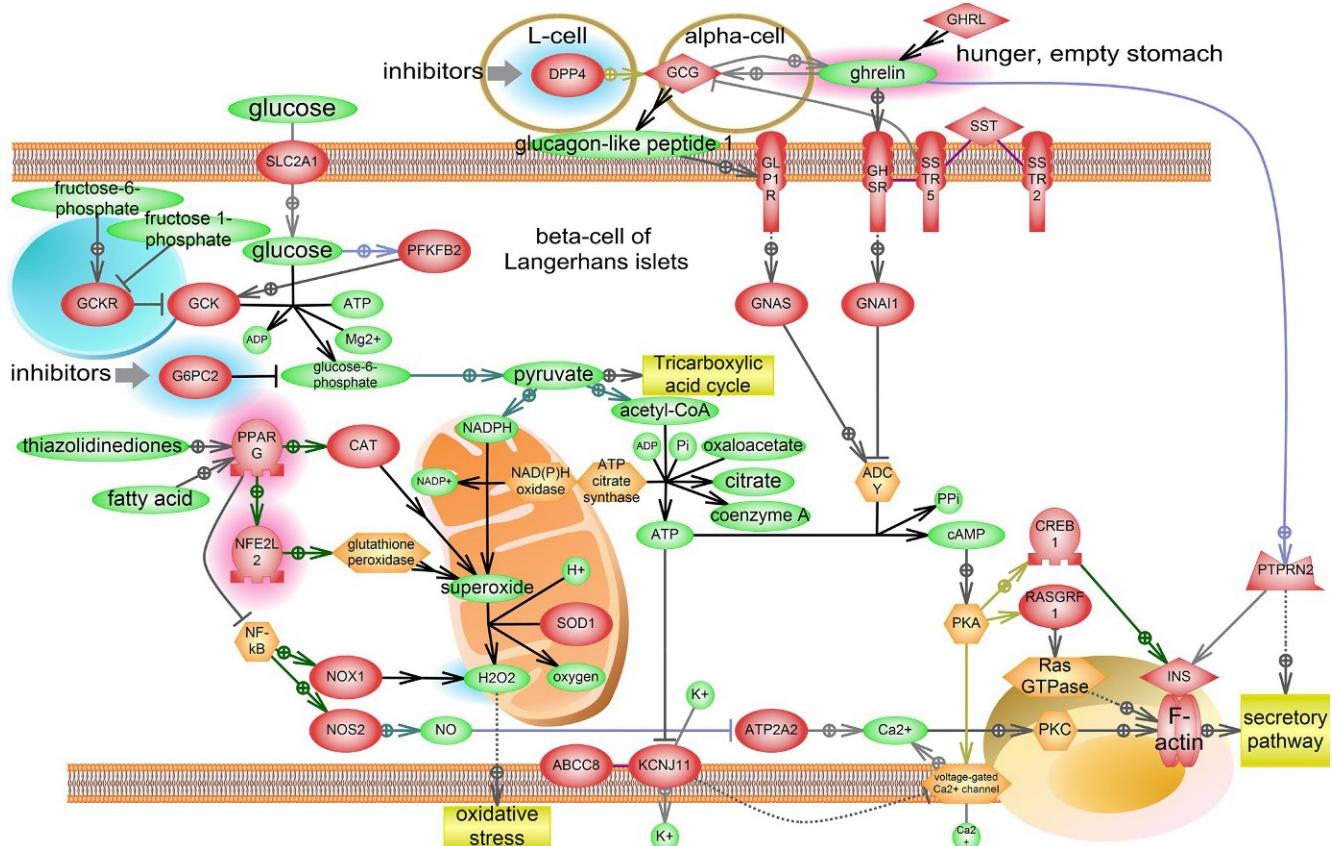


FIG. 9 Pathway 3: Beta-cell proliferation and compensation: Recovery.

## Pathway 4

### Insulin resistance

#### Causes and inductors

Insulin from pancreatic beta cells is an anabolic hormone that reduces the level of circulating glucose by allowing its entry into cells and its subsequent conversion into the storage molecule glycogen that is in turn metabolized during an energy deficiency.

Diabetes mellitus type 2 is characterized by reduced sensitivity to insulin (insulin resistance) in target tissues and by decreased insulin secretion in the final stages of the disease. Insulin resistance occurs in genetically predisposed individuals who cannot adapt by increasing insulin secretion to maintain normoglycemia ([Popa and Mota, 2013](#)).

Disease manifestations begin when insulin-dependent tissues begin to sense a lack of glucose resulting from insulin resistance. Myocytes and hepatocytes are examples of insulin-dependent cells that suffer from insulin resistance because of high levels of adipose tissue in muscles and the liver.

Specifically, in response to a high-fat diet, adipose tissues release substances that include proinflammatory cytokines, chemokines, and free fatty acids that in turn provoke the inhibition of insulin (INS) signal transduction in adjacent specialized cells, thereby leading to the disruption of glycogen and essential protein synthesis.

#### Outcome effects

Insulin resistance is one consequence of obesity and a hallmark of diabetes mellitus type 2.

Local inflammation and elevated levels of circulating fatty acids in obese individuals provoke insulin resistance, causing hepatic steatosis or fatty liver.

High levels of triglycerides or FFA produced through beta-oxidation leads to the accumulation of triglycerides and promotes ongoing gluconeogenesis in the liver resulting in increased levels of glucose in the blood. Despite high blood glucose levels, impaired insulin receptor signaling causes low glucose levels in hepatocytes and muscle cells, thereby disrupting their function.

Adiponectin is a peptide hormone secreted by adipose tissue that prevents insulin resistance in healthy humans by regulating glucose and lipid homeostasis (fatty acid oxidation) ([Wang et al., 2009](#)).

## Signaling

### **Insulin signaling (Fig. 10)**

Firstly, to stimulate glucagon synthesis, signals from the insulin receptor (INSR) are transduced to IRS1/2, which in turn activate PI3K. PI3K-generated PIP3 activates AKT1 leading to the translocation of the GLUT proteins (SLC2A1, SLC2A2, and SLC2A4) into the plasma membrane. GLUT1 (SLC2A1) is the specific glucose transporter responsible for glucose entry into the cell. Once in the cell, glucose is converted into glycogen through several steps. The first step involves glucose phosphorylation by hexokinase. The resulting glucose-6-P undergoes isomerization by phosphoglucomutases. Translocation of a phosphate group to the first position leads to the formation of glucose-1-P. UGP2 converts glucose-1-P to UDP-glucose. The reaction of the last step in the conversion to glycogen involves the addition of UDP-glucose molecules to glycogen chains that is catalyzed by glycogen synthase 2 (GYS2). Insulin signaling can activate GYS2, for example, through inactivation of the GYS2 inhibitor (GSK3B) by AKT1.

Insulin stimulates cell proliferation and protein synthesis by activating the AKT1 and SHC1/MAPK cascades. Only the principal players in these cascades, such as the mammalian target of the rapamycin complex I (mTOR or TORC1), are shown in Fig. 10. Insulin signaling also stimulates glycolysis and increases lipid metabolism in target cells (such as muscle cells and hepatocytes) through FOXO1 and SREBF1 activation (Lizcano and Alessi, 2002; Saltiel and Kahn, 2001; Siddle, 2011).

### **Inflammation-related insulin resistance (Fig. 11)**

The excess levels of triglycerides in adipose tissue caused by a high-fat diet provoke local inflammation. However, the precise molecular mechanism underlying this phenomenon remains elusive (Torres-Leal et al., 2010). Probably, triglycerides facilitate the expression of the chemoattractant chemokine (C-C motif) ligand 2 (CCL2 or MCP1) that in turn recruits macrophages to adipose tissue. Triglycerides may also activate NF- $\kappa$ B-regulated TNF transcription. The binding of this proinflammatory cytokine to its receptor, TNFRSF1A, on the surface of both macrophages and adipocytes leads to the release of IL6 and CXCL5. IL6 provokes insulin resistance in hepatocytes, while CXCL5 does so in muscle cells (Chavey and Fajas, 2009).

Several signaling pathways of insulin resistance that are regulated by inflammation were described. Inactivation of an insulin receptor substrate (IRS1 and IRS2) blocks insulin signaling in hepatocytes/myocytes. Initially, this occurs due to SOCS3 or PTPN1 overexpression that is induced by proinflammatory cytokines such as IL6 or TNF. IL6 through JAK/STAT3 induces SOCS3 expression, and SOCS blocks insulin signaling directly by inhibiting IRS. Conversely, TNF causes the

expression of PTPN1, which in turn dephosphorylates IRS to prevent propagation of the signal. Moreover, cytokines use other branches of the signaling pathways to block IRS. Insulin itself can block its receptor signaling via different feedback mechanisms (Bastard et al., 2006; Shulman, 2000).

### **FFA-related insulin resistance (Fig. 12)**

Insulin promotes lipogenesis, thereby resulting in the storage of triglycerides in adipocytes and of low-density lipoproteins (LDL) in hepatocytes. Insulin stimulates lipogenesis by activating glucose import, regulating the levels of glycerol-3-P and lipoprotein lipase (LPL).

Intracellular fat is formed from free fatty acids (FFA) delivered into cells by lipoproteins and by glycerol-3-P. In the cell, fatty acids are transformed into fatty acyl CoA by ACSL1. Fatty acyl CoA and glycerol-3-P are used to form phosphatidic acid by AGPAT9. Phosphatidic acid is in turn converted into diacylglycerol by phosphatidate phosphatase (not shown). The binding of an additional fatty acyl CoA to diacylglycerol results in the formation of triglycerides. In hepatocytes, triglycerides with phospholipids, cholesterol, and APOB are packaged into very-low-density lipoprotein complexes (VLDL) (Fritsche et al., 2008; Schmitz-Peiffer, 2000).

Triglycerides stored in adipose tissue are destructed by PNPLA2 and LIPE. FABP4 transports the released FFA to the cell membrane and SLC27A1 exports them from the cell.

Increased blood levels of FFA eventually provoke insulin resistance in peripheral tissues (Nagle et al., 2009). The precursor to triglycerides, diacylglycerol, can itself activate protein kinase C theta (PRKCQ), which phosphorylates IRS1 and blocks insulin signal transduction. Free fatty acids bind to the toll-like receptors (TLR2 and TLR4) and through MYD88/IRAK/TRAF6 they activate MAPKs and NF- $\kappa$ B and they phosphorylate IRS, thereby inactivating it.

The fatty acids that escape oxidation may be converted into triglycerides with the ability to trigger insulin resistance. Decreased levels of adiponectin (ADIPOQ) can lead to the escape of fatty acids from oxidation in adipocytes or hepatocytes. Usually, ADIPOQ modulates some metabolic processes that include glucose regulation and fatty acid oxidation. ADIPOQ acts through its receptors ADIPOR1 and ADIPOR2. Upon binding to ADIPOR2, the hormone induces the expression of peroxisome proliferator-activated receptor alpha (PPARA), which promotes the transcription of lipoprotein lipase (LPL), acyl-CoA oxidase 1 (ACOX1), and other genes and thus stimulates fatty acid oxidation.

The ADIPOR1 receptor interacts with an adaptor protein (APPL1) to transduce the signal from adiponectin to the AMP-dependent protein kinases (AMPK), which in turn negatively regulate the transcription of

proteins involved in gluconeogenesis. Activated APPL1, acting through AKT1 and the MAPK cascade, promotes the translocation of GLUT4 (SLC2A4) into the cell membrane where it is responsible for glucose intake. Intracellular glucose is converted into dihydroxyacetone-P during glycolysis and is then transformed into glycerol-3-P by glycerol-3-phosphate dehydrogenase ( $\text{NAD}^+$ ) to then participate in fatty oxidation. In hepatocytes, glycerol-3-P may also be produced from the glycerol released from lipoproteins by glycerol kinase.

Lower levels of the ADIPOQ hormone are inversely correlated with body fat percentage in adults. ADIPOQ is exclusively secreted from adipose tissue. Local inflammation and the proinflammatory cytokines TNF and IL6 may decrease ADIPOQ transcription through interactions between FOXO1 and the peroxisome proliferator-activated receptor gamma protein (PPARG). A high-fat diet may inhibit ADIPOQ transcription by involving the negative adiponectin transcription regulators ATF3 (activating transcription factor 3) and NFATC4 ([Deepa and Dong, 2009](#); [Shehzad et al., 2012](#)).

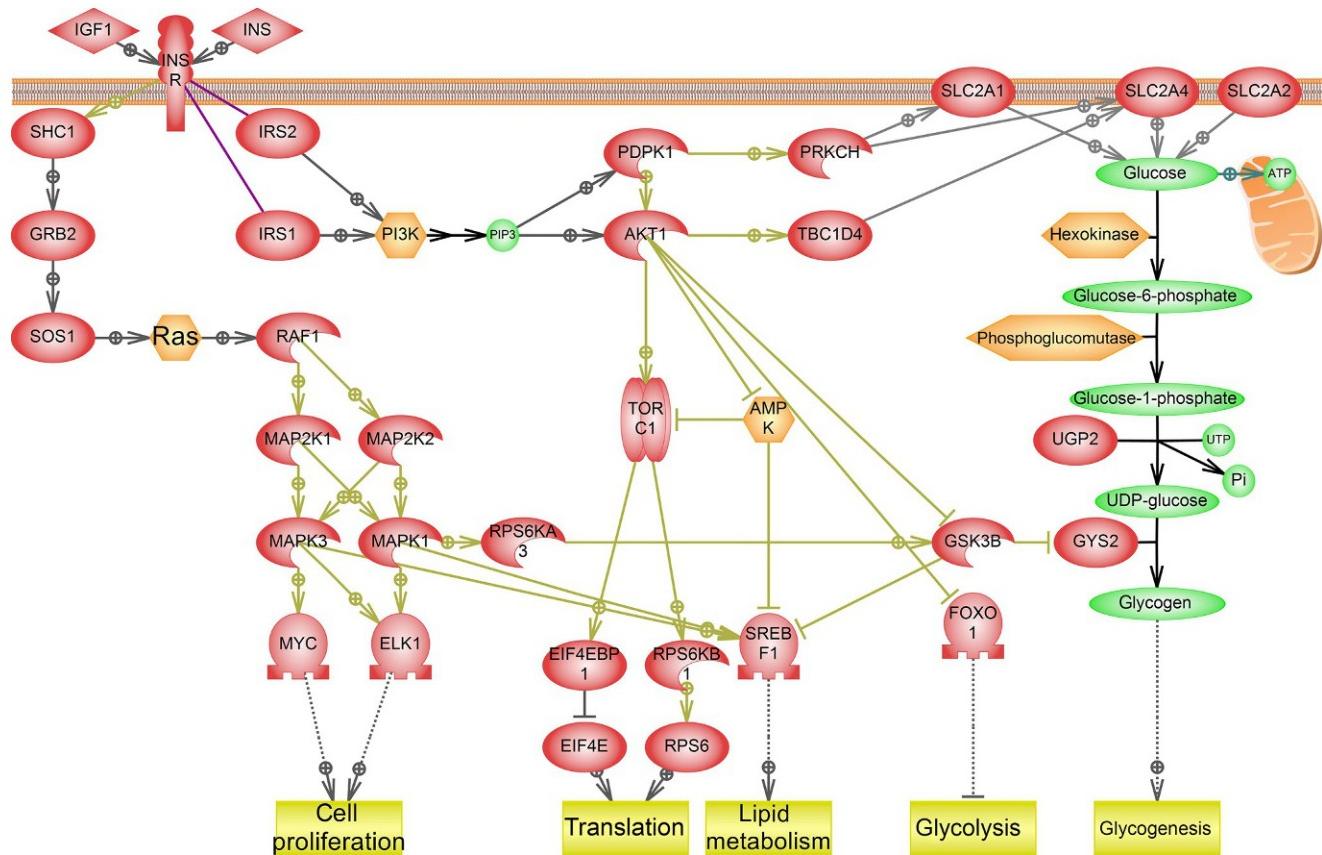
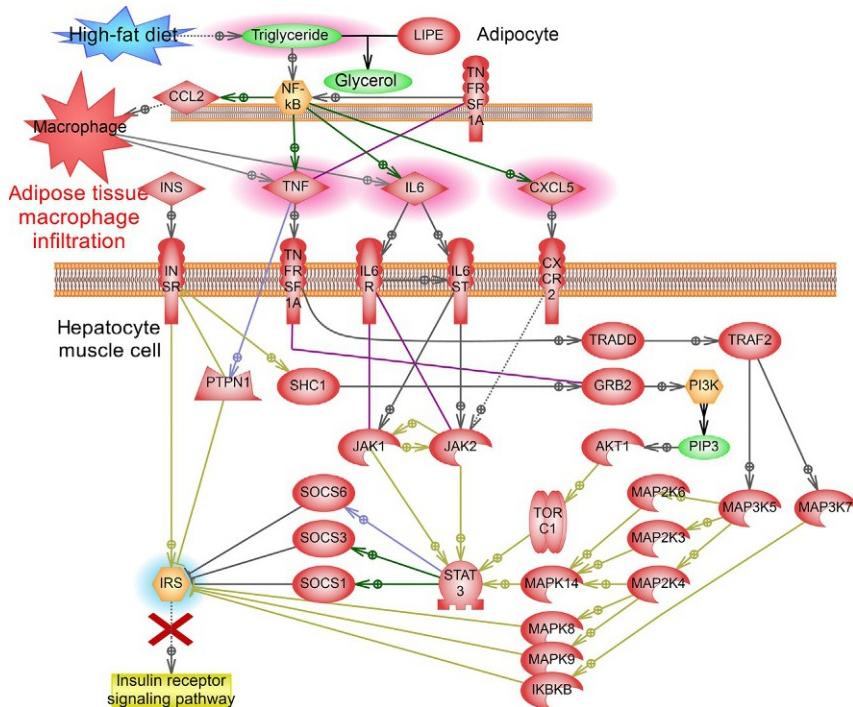
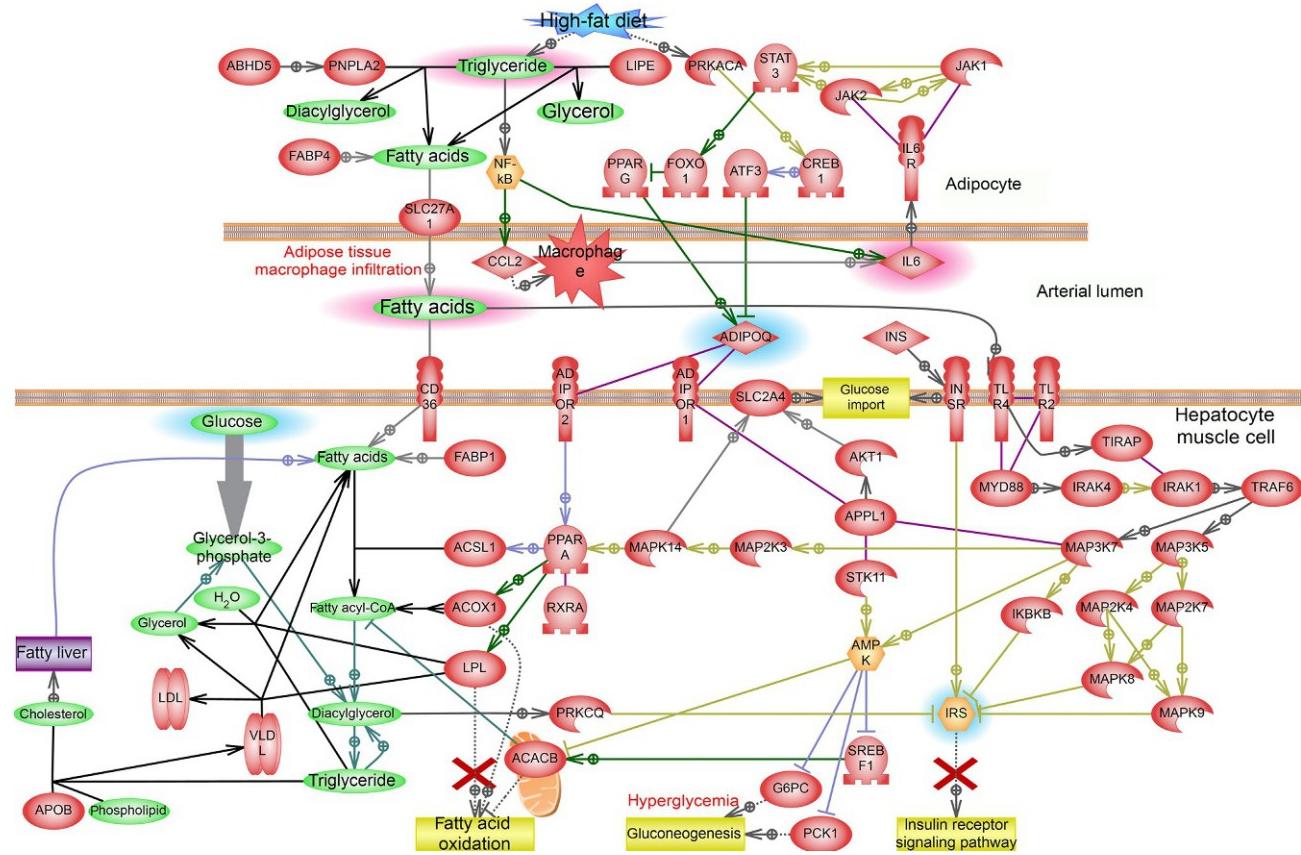


FIG. 10 Pathway 4: Insulin resistance: Insulin signaling.



**FIG. 11** Pathway 4: Insulin resistance: Inflammation-related insulin resistance.

## II. Human disease pathways



**FIG. 12** Pathway 4: Insulin resistance: FFA-related insulin resistance.

## References

- Disease numbers # 222100 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: E11. (Endocrine, nutritional and metabolic diseases (E00-E90)).
- Alarcón, C., Lincoln, B., Rhodes, C.J., 1993. The biosynthesis of the subtilisin-related proprotein convertase PC3, but not that of the PC2 convertase, is regulated by glucose in parallel to proinsulin biosynthesis in rat pancreatic islets. *J. Biol. Chem.* 268, 4276–4280.
- Alismail, H., Jin, S., 2014. Microenvironmental stimuli for proliferation of functional islet  $\beta$ -cells. *Cell Biosci.* 4, 12. <https://doi.org/10.1186/2045-3701-4-12>.
- Amisten, S., Salehi, A., Rorsman, P., Jones, P.M., Persaud, S.J., 2013. An atlas and functional analysis of G-protein coupled receptors in human islets of Langerhans. *Pharmacol. Ther.* 139, 359–391. <https://doi.org/10.1016/j.pharmthera.2013.05.004>.
- Andrali, S.S., Sampley, M.L., Vanderford, N.L., Özcan, S., 2008. Glucose regulation of insulin gene expression in pancreatic  $\beta$ -cells. *Biochem. J.* 415, 1–10. <https://doi.org/10.1042/BJ20081029>.
- Ashcroft, F.M., 2005. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J. Clin. Invest.* 115, 2047–2058. <https://doi.org/10.1172/JCI25495>.
- Bastard, J.-P., Maachi, M., Lagathu, C., Kim, M.J., Caron, M., Vidal, H., Capeau, J., Feve, B., 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine Netw.* 17, 4–12.
- Cao, M., Tong, Y., Lv, Q., Chen, X., Long, Y., Jiang, L., Wan, J., Zhang, Y., Zhang, F., Tong, N., 2012. PPAR $\delta$  activation rescues pancreatic  $\beta$ -cell line INS-1E from Palmitate-induced endoplasmic reticulum stress through enhanced fatty acid oxidation. *PPAR Res.* 2012, 680684. <https://doi.org/10.1155/2012/680684>.
- Chavey, C., Fajas, L., 2009. CXCL5 drives obesity to diabetes, and further. *Aging* 1, 674–677. <https://doi.org/10.1863/aging.100064>.
- Cnop, M., Welsh, N., Jonas, J.-C., Jörns, A., Lenzen, S., Eizirik, D.L., 2005. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. *Diabetes* 54 (Suppl. 2), S97–107.
- Collombat, P., Xu, X., Ravassard, P., Sosa-Pineda, B., Dussaud, S., Billestrup, N., Madsen, O.D., Serup, P., Heimberg, H., Mansouri, A., 2009. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into  $\alpha$  and subsequently  $\beta$  cells. *Cell* 138, 449–462. <https://doi.org/10.1016/j.cell.2009.05.035>.
- Cullen, K.S., Al-Oanzi, Z.H., O'Harte, F.P.M., Agius, L., Arden, C., 2014. Glucagon induces translocation of glucokinase from the cytoplasm to the nucleus of hepatocytes by transfer between 6-phosphofructo 2-kinase/fructose 2,6-bisphosphatase-2 and the glucokinase regulatory protein. *Biochim. Biophys. Acta Mol. Cell Res.* 1843, 1123–1134. <https://doi.org/10.1016/j.bbamcr.2014.02.006>.
- Deepa, S.S., Dong, L.Q., 2009. APPL1: role in adiponectin signaling and beyond. *Am. J. Physiol. Endocrinol. Metab.* 296, E22–E36. <https://doi.org/10.1152/ajpendo.90731.2008>.
- Delporte, C., 2013. Structure and physiological actions of ghrelin. *Scientifica* 2013, 1–25. <https://doi.org/10.1155/2013/518909>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fonseca, V.A., 2009. Defining and characterizing the progression of type 2 diabetes. *Diabetes Care* 32, S151–S156. <https://doi.org/10.2337/dc09-S301>.
- Fritzsche, L., Weigert, C., Häring, H.-U., Lehmann, R., 2008. How insulin receptor substrate proteins regulate the metabolic capacity of the liver—implications for health and disease. *Curr. Med. Chem.* 15, 1316–1329.
- Fujita, Y., Haneda, M., 2011. Molecular mechanism of insulin secretion facilitated by incretin. *Nihon Rinsho Jpn. J. Clin. Med.* 69, 808–812.

- Habener, J.F., Stanojevic, V., 2012.  $\alpha$ -cell role in  $\beta$ -cell generation and regeneration. *Islets* 4, 188–198. <https://doi.org/10.4161/isl.20500>.
- Henquin, J.C., 2000. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 49, 1751–1760.
- Johnson, J.D., Luciani, D.S., 2010. Mechanisms of pancreatic  $\beta$ -cell apoptosis in diabetes and its therapies. In: Islam, M.S. (Ed.), *The Islets of Langerhans*. Springer Netherlands, Dordrecht, pp. 447–462. [https://doi.org/10.1007/978-90-481-3271-3\\_19](https://doi.org/10.1007/978-90-481-3271-3_19).
- Kaiser, N., Leibowitz, G., 2009. Failure of beta-cell adaptation in type 2 diabetes: lessons from animal models. *Front. Biosci. (Landmark Ed.)* 14, 1099–1115.
- Lizcano, J.M., Alessi, D.R., 2002. The insulin signalling pathway. *Curr. Biol.* 12, R236–R238.
- Marrif, H.I., Al-Sunousi, S.I., 2016. Pancreatic  $\beta$  cell mass death. *Front. Pharmacol.* 7. <https://doi.org/10.3389/fphar.2016.00083>.
- Nagle, C.A., Klett, E.L., Coleman, R.A., 2009. Hepatic triacylglycerol accumulation and insulin resistance. *J. Lipid Res.* 50 (Suppl), S74–S79. <https://doi.org/10.1194/jlr.R800053-JLR200>.
- Park, S., Jiang, H., Zhang, H., Smith, R.G., 2012. Modification of ghrelin receptor signaling by somatostatin receptor-5 regulates insulin release. *Proc. Natl. Acad. Sci.* 109, 19003–19008. <https://doi.org/10.1073/pnas.1209590109>.
- Pillay, K., Govender, P., 2013. Amylin uncovered: a review on the polypeptide responsible for type II diabetes. *Biomed. Res. Int.* 2013, 1–17. <https://doi.org/10.1155/2013/826706>.
- Poitout, V., Hagman, D., Stein, R., Artner, I., Robertson, R.P., Harmon, J.S., 2006. Regulation of the insulin gene by glucose and fatty acids. *J. Nutr.* 136, 873–876.
- Popa, S., Mota, M., 2013. Beta-cell function and failure in Type 2 diabetes. In: Masuo, K. (Ed.), *Type 2 Diabetes*. <https://www.intechopen.com/books/type-2-diabetes/beta-cell-function-and-failure-in-type-2-diabetes>, <https://doi.org/10.5772/56467>.
- Pound, L.D., Oeser, J.K., O'Brien, T.P., Wang, Y., Faulman, C.J., Dadi, P.K., Jacobson, D.A., Hutton, J.C., McGuinness, O.P., Shiota, M., O'Brien, R.M., 2013. G6PC2: a negative regulator of basal glucose-stimulated insulin secretion. *Diabetes* 62, 1547–1556. <https://doi.org/10.2337/db12-1067>.
- Puddu, A., Sanguineti, R., Mach, F., Dallegri, F., Viviani, G.L., Montecucco, F., 2013. Update on the protective molecular pathways improving pancreatic Beta-cell dysfunction. *Mediat. Inflamm.* 2013, 1–14. <https://doi.org/10.1155/2013/750540>.
- Sachdeva, M.M., Stoffers, D.A., 2009. Minireview: Meeting the demand for insulin: molecular mechanisms of adaptive postnatal  $\beta$ -cell mass expansion. *Mol. Endocrinol.* 23, 747–758. <https://doi.org/10.1210/me.2008-0400>.
- Saltiel, A.R., Kahn, C.R., 2001. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 414, 799–806. <https://doi.org/10.1038/414799a>.
- Schmitz-Peiffer, C., 2000. Signalling aspects of insulin resistance in skeletal muscle: mechanisms induced by lipid oversupply. *Cell. Signal.* 12, 583–594.
- Shao, S., Fang, Z., Yu, X., Zhang, M., 2009. Transcription factors involved in glucose-stimulated insulin secretion of pancreatic beta cells. *Biochem. Biophys. Res. Commun.* 384, 401–404. <https://doi.org/10.1016/j.bbrc.2009.04.135>.
- Shao, S., Yang, Y., Yuan, G., Zhang, M., Yu, X., 2013. Signaling molecules involved in lipid-induced pancreatic beta-cell dysfunction. *DNA Cell Biol.* 32, 41–49. <https://doi.org/10.1089/dna.2012.1874>.
- Shehzad, A., Iqbal, W., Shehzad, O., Lee, Y.S., 2012. Adiponectin: regulation of its production and its role in human diseases. *Hormones (Athens, Greece)* 11, 8–20.
- Shulman, G.I., 2000. Cellular mechanisms of insulin resistance. *J. Clin. Invest.* 106, 171–176. <https://doi.org/10.1172/JCI10583>.
- Siddle, K., 2011. Signalling by insulin and IGF receptors: supporting acts and new players. *J. Mol. Endocrinol.* 47, R1–10. <https://doi.org/10.1530/JME-11-0022>.

- Soleimaniour, S.A., Crutchlow, M.F., Ferrari, A.M., Raum, J.C., Groff, D.N., Rankin, M.M., Liu, C., De León, D.D., Naji, A., Kushner, J.A., Stoffers, D.A., 2010. Calcineurin signaling regulates human islet  $\beta$ -cell survival. *J. Biol. Chem.* 285, 40050–40059. <https://doi.org/10.1074/jbc.M110.154955>.
- Szabat, M., Lynn, F.C., Hoffman, B.G., Kieffer, T.J., Allan, D.W., Johnson, J.D., 2012. Maintenance of  $\beta$ -cell maturity and plasticity in the adult pancreas: developmental biology concepts in adult physiology. *Diabetes* 61, 1365–1371. <https://doi.org/10.2337/db11-1361>.
- Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S., Herrera, P.L., 2010. Conversion of adult pancreatic  $\alpha$ -cells to  $\beta$ -cells after extreme  $\beta$ -cell loss. *Nature* 464, 1149–1154. <https://doi.org/10.1038/nature08894>.
- Torres-Leal, F.L., Fonseca-Alaniz, M.H., Rogero, M.M., Tirapegui, J., 2010. The role of inflamed adipose tissue in the insulin resistance. *Cell Biochem. Funct.* 28, 623–631. <https://doi.org/10.1002/cbf.1706>.
- Uchizono, Y., Alarcón, C., Wicksteed, B.L., Marsh, B.J., Rhodes, C.J., 2007. The balance between proinsulin biosynthesis and insulin secretion: where can imbalance lead? *Diabetes Obes. Metab.* 9 (Suppl. 2), 56–66. <https://doi.org/10.1111/j.1463-1326.2007.00774.x>.
- Wang, Y., Zhou, M., Lam, K.S.L., Xu, A., 2009. Protective roles of adiponectin in obesity-related fatty liver diseases: mechanisms and therapeutic implications. *Arq. Bras. Endocrinol. Metabol.* 53, 201–212.

## CHAPTER

## 4.3

## Diabetic neuropathy

Diabetic neuropathy (DN) is the most common chronic complication of diabetes mellitus with diverse clinical manifestations. About 50% of patients with long-duration diabetes suffer from DN. DN can develop in patients with prediabetes and in patients with either type 1 or type 2 diabetes. However, the mechanisms underlying the pathogenesis of DN have not been completely clarified. Patients with poor glycemic control, diabetic nephropathy, or retinopathy are at increased risk, and their prognosis is unfavorable.

Diabetic polyneuropathy (Diabetic peripheral neuropathy) is a distal symmetric polyneuropathy characterized by numbness, tingling, pain, or weakness that affects the nerves in a stocking-and-glove pattern, beginning in the distal extremities. Diabetic polyneuropathy leads to substantial pain, morbidity, and impaired quality of life. (*Ferri and Ferri, 2018*).

Nerve damage begins long before the first symptoms of diabetic polyneuropathy appear. Moreover, some patients with DN may have no symptoms so a careful medical examination may be needed to identify the disorder. The classification of diabetic neuropathies is complex based on the diversity in pathology, symptoms, clinical course, etiology, and the pattern of neurological damage. The different DN forms can be categorized in terms of their anatomical distribution (e.g., proximal or distal, symmetric or asymmetric, and focal or multifocal or diffuse), clinical state (e.g., acute, subacute, or chronic), clinical symptoms (painful or nonpainful), or pathophysiology (sensory, motor, or autonomic). Classification into “typical” or “atypical” forms is based on compliance with minimal criteria that were suggested for typical DN.

DN symptoms vary depending on the types of nerve fibers involved. Both small myelinated and unmyelinated nerve fibers as well as large myelinated nerve fibers are involved in DN pathogenesis and progression. Damage to large fibers causes proprioception (the sense of orientation of the body) impairment. Injury to small fibers deteriorates the perception of temperature and pain (nociception) leading to paresthesias (dermal feelings without real physical cause, e.g., the pricking, numbness,

chilling, and burning), dysesthesias (unpleasant feeling of touch), and neuropathic pain. In most cases, lesions of small nerve fibers happen first. Advanced neuropathy may be combined with other complications such as ulceration and neuroarthropathy (Charcot's joints) of the foot ([Albers and Pop-Busui, 2014](#); [Ang et al., 2018](#); [Shakher and Stevens, 2011](#)).

The most typical forms of DN include distal symmetrical sensory-motor polyneuropathy (DSPN) and diabetic autonomic neuropathy (DAN) that are usually chronic and often accompany advanced disease. DSPN is a particularly painful peripheral diabetic neuropathy that is characterized by numbness, tingling, pain, or weakness and is associated with poor quality of life. In DSPN, sensory deficits include dysfunction of superior motor nerves that appears first in the distal parts of the extremities and are termed "stocking-glove" signs, which then progresses proximally. DAN, which is often followed by DSPN, can affect any sympathetic or parasympathetic autonomic functions. The clinical symptoms of DAN may be non-specific. Therefore, DAN is a poorly diagnosed complication of diabetes.

Diabetic neuropathy is a multifunctional disorder with highly complex mechanisms of pathogenesis that are not completely understood.

Nerve fibers are long projections of neurons covered by a sheath of Schwann glial cells and by endoneurium formed by connective tissue. The sheaths of myelinated nerve fibers include the protein myelin, which is produced by Schwann cells. To better conduct signals, nerve fibers are assembled in bundles that connect with microvessels by way of the perineurium.

The chronic hyperglycemia characteristic of diabetes causes damage to the microvessels (microangiopathy), resulting in impaired nutrition of peripheral nerve fibers. The limited vascular supply to peripheral nerves also makes nerves fibers susceptible to ischemia/hypoxia. Damage to the perineurium microvessels is likely the most critical factor in neuronal injury and dysfunction in DN:

**Pathway 1. Microangiopathy in diabetic neuropathy (Fig. 13).**

Microangiopathy itself does not reveal the full complexity of DN pathogenic mechanisms. The signaling pathways directly triggered by hyperglycemia and hypoxia are similar in different cell types although they demonstrate some cellular specificity. In neurons, hyperglycemia induces apoptosis and ER stress, and it alters autophagy.

**Pathway 2. Neuron and Schwann cell death (Fig. 14).**

In general the mechanisms promoting pain in DN are poorly understood. There is increasing evidence that alterations to the somatosensory nervous system drive sustained pain. Changes in nociceptors located in the skin and the associated activation of the brain leads to neuropathic pain.

**Pathway 3. Pain perception in diabetic neuropathy (Fig. 15).**

## Key cellular contributors and processes

Hyperglycemia

Process

Hyperglycemia refers to an abnormally high blood sugar level, and it is a hallmark of diabetes.

Hypoxia

Process

Hypoxia is an abnormally low oxygen level in a tissue or organ.

Ischemia

Process

Ischemia is the restricted blood supply to a tissue or organ caused by an obstruction or the narrowing of a blood vessel.

Osmolarity

Process

Osmolarity or osmotic concentration is the solute concentration defined as the number of osmoles of solute per liter of solution.

## Pathway 1

### Microangiopathy in diabetic neuropathy (Fig. 13)

#### Incoming signals

Hyperglycemia and the accumulation of advanced glycation end products (AGEs) result in multiple effects leading to microangiopathy. In DN, hyperglycemia triggers the alternative pathways of glucose utilization, oxidative stress, glycation, and energetic defects in the cells of microvessels.

Glycation is a nonenzymatic chemical modification of the structure and function of proteins, lipids, and nucleic acids that involves the addition of reactive carbohydrate groups to those molecules. Accumulated advanced glycation end products (AGEs), reactive oxygen species (ROS), and products of alternative metabolic pathways damage endothelial cells form thickened basal membranes, trigger occlusion and constriction of the microvessels, and violate their permeability. Further, AGEs bind to their receptors on macrophages and stimulate the production of inflammatory cytokines.

#### Outcome effects

Eventually, microangiopathy leads to the disruption of nerve perfusion and ischemia. Similar to processes of alternative glucose utilization, glycation, oxidative stress, and the energetic defects that occur in the cells of microvessels also take place in Schwann cells and neurons leading to neuronal dysfunction. Diabetic microangiopathy is also responsible for the development of ulcers of peripheral tissues, diabetic retinopathy, and diabetic nephropathy.

#### Signaling

Excessively high levels of intracellular glucose activate the polyol (sorbitol) and hexose pathways.

In the polyol pathway, excess glucose is transformed into sorbitol by aldose reductase (AKR1B1) using NADPH as a cofactor. Sorbitol dehydrogenase (SORD) can oxidize sorbitol to form fructose and produce NADH. This process leads to the accumulation of NADH and sorbitol and the depletion of NADPH in affected cells. High levels of sorbitol induce hyperosmotic stress in cells.

Excess NADH contributes to the generation of reactive oxygen species (ROS). On the other hand, the lack of NADPH, due to its consumption by AKR1B1, causes reduced glutathione production by glutathione

reductase (GSR). High levels of ROS and of reduced glutathione deplete the cell's ability to respond to oxidative stress.

Fructose produced by the polyol pathway is phosphorylated to form fructose-3-phosphate and fructose-6-phosphate, which are in turn broken down into 3-deoxyglucosone and methylglyoxal. Both of these compounds are powerful glycosylating agents that are involved in the formation of AGEs.

Extracellular AGEs activate the advanced glycosylation end product-specific receptor (AGER) that stimulates MAPK cascade and AKT1/NFKB signaling via PI3K. Activation of these pathways triggers the expression of several different factors such as VEGFA, TGFB1, the adhesion molecules ICAM1 and VCAM1, the interleukins IL-6 and IL-1A, SERPINE1, and endothelin 1 (EDN1). TGFB1 and VEGFA induce blood vessel permeability and stimulate endothelial cell proliferation, EDN1 is responsible for vasoconstriction, and SERPINE1 inhibits the plasminogen activators PLAT and PLA2, thereby disrupting the process of blood clotting. ICAM1, VCAM1, and the interleukins mediate leukocyte-endothelial cell adhesion and macrophage chemotaxis, which together promote inflammation.

Excess glucose can also be shunted into the hexose pathway in which fructose-6-phosphate is converted to uridine-diphosphate-*N*-acetylglucosamine (UDP-GlcNAc).

Initially, glucose-6-phosphate is converted to fructose-6-phosphate by glucose-6-phosphate isomerase (GPI). Glutamine-fructose-6-phosphate transaminase 1 (GFPT1) then catalyzes the conversion of fructose-6-phosphate to glucosamine-6-phosphate. Glucosamine-phosphate *N*-acetyltransferase 1 (GNPNAT1) then converts that to *N*-acetyl-glucosamine-6-phosphate (GlcNAc6P). Finally the phosphoglucomutase 3 (PGM3) transforms GlcNAc6P to *N*-acetyl-glucosamine-1-phosphate, which is in turn converted to UDP-GlcNAc by UDP-*N*-acetylglucosamine pyrophosphorylase 1 (UAP1) (Edwards et al., 2008; Figueroa-Romero et al., 2008; Vincent et al., 2011; Yagihashi et al., 2011).

UDP-GlcNAc stimulates the transcription factor SP1, which regulates the expression of PARP1, a nuclear enzyme associated with oxidative stress, metabolic derangements, and cell death (see *Pathway 3*).

Increased intracellular levels of glucose also stimulate the formation of diacylglycerol that in turn activates AKT1/NFKB signaling.

## II. Human disease pathways

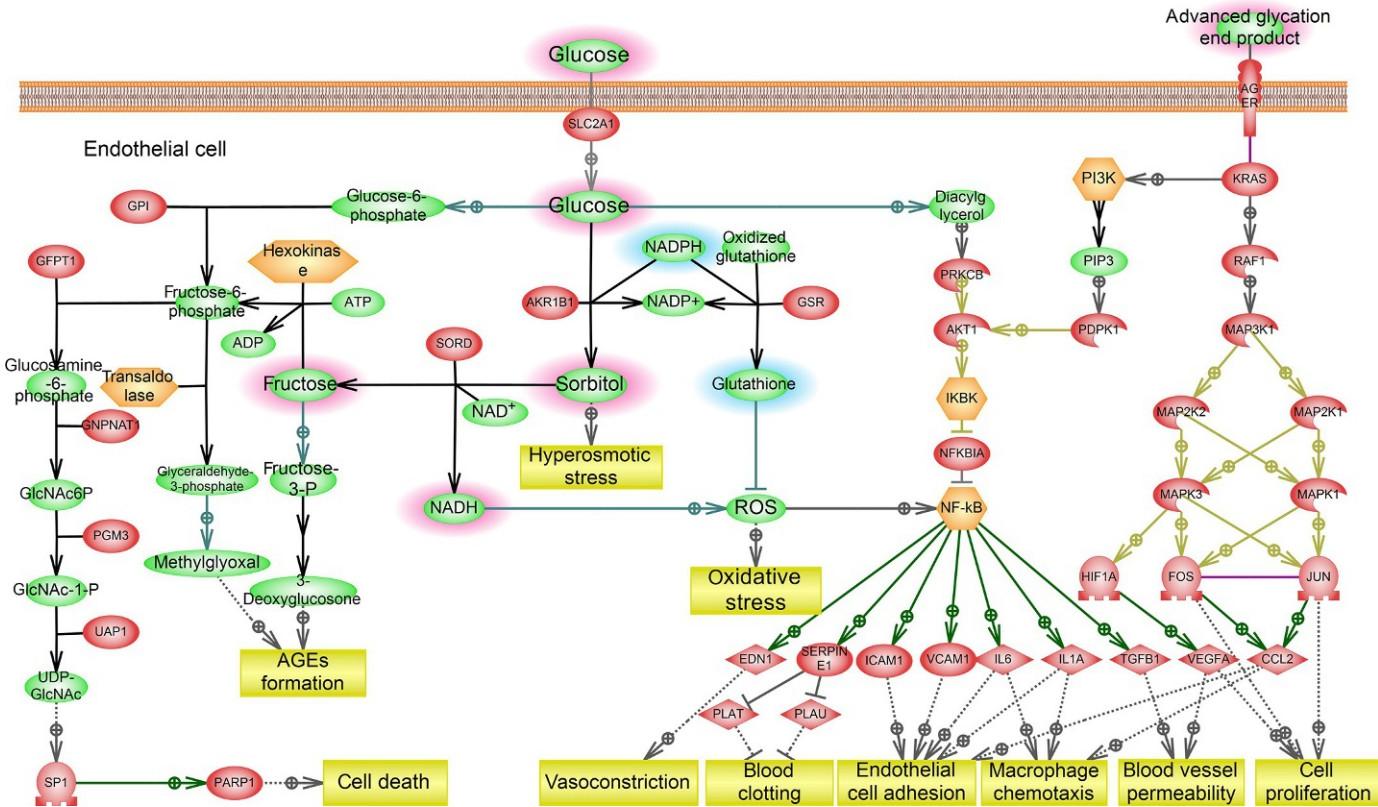


FIG. 13 Pathway 1: Microangiopathy in diabetic neuropathy.

## Pathway 2

### Neuron and Schwann cell death (Fig. 14)

#### Incoming signals

Hypoglycemia-related hypoxia, advanced glycosylation end products, and elevated cytokines levels cause the death of Schwann cells and neurons.

Autophagy, necrosis, and apoptosis are the most common types of cellular death observed. Autophagy is the intracellular degradation of damaged organelles and protein aggregates by lysosomes. Apoptosis is a highly controlled process activated either via plasma membrane receptors or via permeabilization of the mitochondria. Necrosis is a mostly uncontrolled process leading to mitochondrial swelling and cell rupture leading to inflammation reaction. States of  $\text{Ca}^{2+}$  overload and energy deficiency trigger necrosis. Changes in mitochondrial morphology play an essential role in both neuronal apoptosis and necrosis.

#### Outcome effects

Autophagy is usually a protective process; however, such extreme conditions as high glucose levels induce uncontrolled autophagy. The imbalance between autophagy and apoptosis may facilitate the progression of neuronal injury.

Autophagy and endoplasmic reticulum (ER) stress modify the composition of Schwann cell membranes leading to their demyelination and subsequent nerve injury. Persistent ER stress results in neuronal and Schwann cell apoptosis.

#### Signaling

Hypoxia, TNF, and AGEs activate apoptosis in neurons through receptors on their plasma membranes. The advanced glycosylation end product-specific receptor (AGER), toll-like receptors (TLR2 and TLR4) and tumor necrosis factor receptors (TNFRSF1A and TNFRSF1B) trigger the expression of genes through the MYD88, TIRAP, and TRAF proteins. As a result, the activated MAPK cascade and the transcription factor NF- $\kappa$ B stimulate the expression of both the proapoptotic DIABLO and the antiapoptotic mitochondrial BIRC<sub>s</sub>/XIAP complex. Then, MAPK8,9,10 (JNK1/2/3) signaling phosphorylates the antiapoptotic proteins BCL2 and BCL2L1, thus inactivating their functions. There are other players that regulate apoptosis. However, the balance in diabetic neurons is shifted toward the activation of the proapoptotic regulator BAX. BAX induces

the formation of the mitochondrial transition pore, the subsequent release of cytochrome C into the cytoplasm, and the activation of the apoptotic caspases. BAX also induces DIABLO release from mitochondria.

Schwann cells play a significant role in nerve myelination and are incredibly sensitive to alterations in membrane structure. High glucose stress and hypoxia cause the accumulation of misfolded proteins within both the ER and membrane vesicles resulting in activation of the unfolded protein response and changes in the composition of neuronal membranes.

The ER is a membranous network that is required for protein packaging, lipid biosynthesis, and intracellular calcium storage. All cell proteins must undergo specific posttranslational modifications and protein folding within the ER before they are fully functional. High levels of glucose and ROS may dysregulate enzymes and normal chaperone function involved with protein folding. As a result, ER stress develops, and unfolded or misfolded proteins accumulate within the ER.

Unfolded proteins attract chaperone HSPA5 that plays an essential role in protein quality control within the endoplasmic reticulum lumen. Direct targets of HSPA5, such as EIF2AK3, ATF6, and ERN1, are responsible for protein and lipid degradation and for the expression of proapoptotic genes. Unfolded proteins also induce the ATF4-mediated upregulation of DDT3 and the expression of ERO1A, which in turn activates the ER calcium channel ITPR1, resulting in increased levels of cytosolic  $\text{Ca}^{2+}$ . Calcium overload promotes ER stress and impairs the function of mitochondrial cytochrome c oxidase leading to a reduction of ATP levels. Decreased ATP levels cause necrosis.

Finally, activated AGER and high glucose levels trigger AKT/MTOR signaling in neurons. In a healthy cell, MTOR inhibits the kinases ULK1/2, which leads to a delay in the initiation of autophagy. ATP depletion and  $\text{Ca}^{2+}$  overload together activate the cellular energy sensor AMPK that suppresses MTOR and thereby promotes autophagy (Dewanjee et al., 2018; O'Brien et al., 2014; Sifuentes-Franco et al., 2017; Volpe et al., 2018).

## II. Human disease pathways

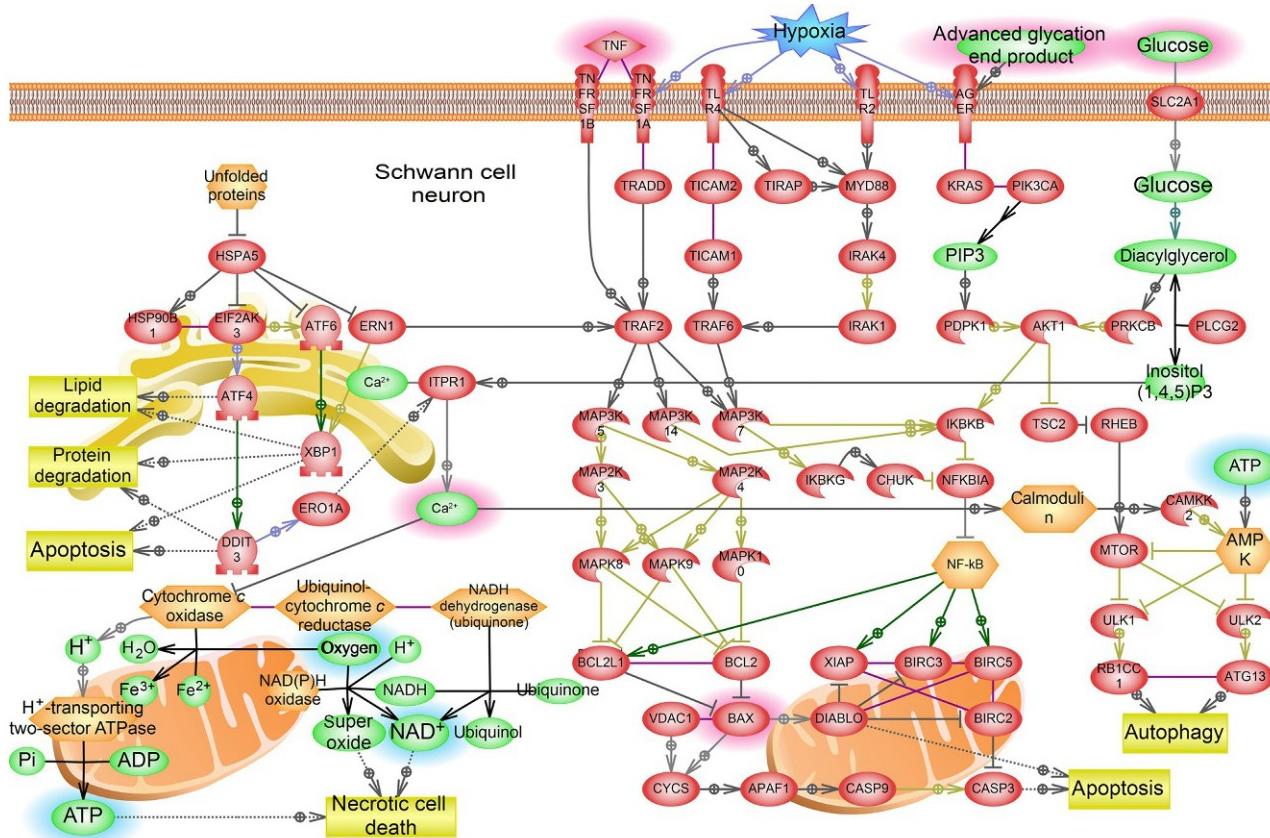


FIG. 14 Pathway 2: Neuron and Schwann cell death.

## Pathway 3

### Pain perception in diabetic neuropathy (Fig. 15)

#### Incoming signals

Pain perception is a complex physiological process. The pathogenesis of pain development may include the pathological activity of damaged nerve fibers, specifically demyelination, the sensitization of pain receptors (nociceptors), the adhesion of peripheral sensory fibers due to their degeneration, and general central nervous system sensitization.

The free endings of nerve fibers react to the pressure, temperature, or chemical stimuli ( $H^+$ ,  $K^+$ , or hormones) that appear when tissue is damaged. The activation of nociceptors is the first stage of pain perception. The bioelectric pain-stimulating signals generated in nociceptors travel to sensory neurons in the dorsal root ganglia of peripheral nerve fibers, enter the spinal cord, switch to subsequent neurons, and activate the brain along spinothalamic tracts. The spinothalamic tracts that pass through the thalamus and partly through the limbic system of the brain control emotions and behavioral reactions. Sensing pain involves the cerebral cortex. Sensory areas of the cerebral cortex are the highest modulators of pain sensitivity and duration, and they serve to localize the origin of the pain impulse.

#### Outcome effects

Diabetes causes permanent activation of membrane-based ion channels that transmit the electrochemical gradient of ions that serves as a nervous impulse to sensory neurons.

Chronic pain causes constant nervous excitation and central nervous system sensitization. Thus minor pain in DN may trigger a disproportionately significant perception. Inhibitory interneurons and descending modulatory control systems lose their activity in DN due to nerve damage.

#### Signaling

$Na^+$ ,  $K^+$ ,  $Cl^-$ , and  $Ca^{2+}$  channels and a number of receptors control the transmission of pain signals in the central nervous system. Expression of the transient receptor potential cation (TRP) channel and the voltage-gated sodium (SCN8A, SCN9A, and SCN10A) and calcium channels (CACNA1A, CACNA1B, and CACNA1H) in the dorsal root ganglion neurons have a significant role in the formation of nociceptive sensation and peripheral nerve sensitization.

Glutamate is the primary neurotransmitter in nerve fibers. Glutamate excites postsynaptic glutamatergic receptors (NMDA-R and AMPA-R) resulting in  $\text{Ca}^{2+}$  and Na influx into the postsynaptic cell and K efflux from the cell. Within the cell, the Na current causes membrane depolarization.

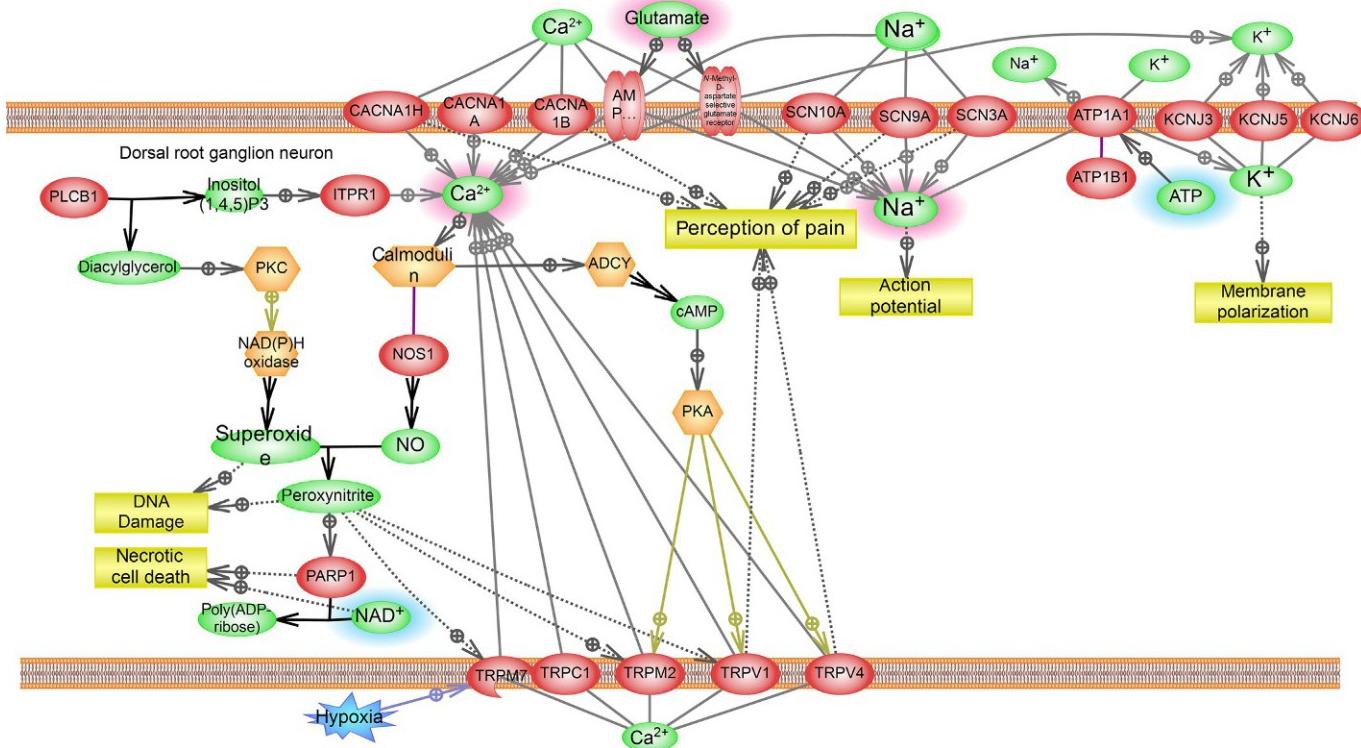
$\text{Ca}^{2+}$  influx stimulates neuronal death via the synthesis of NO.  $\text{Ca}^{2+}$  activates nitric oxide synthase (NOS1) through calmodulin and PKA via calmodulin/ADCY/cAMP. NOS1 catalyzes the production of nitric oxide (NO) that reacts with superoxide to form a toxic free radical peroxynitrite that in turn direct damages intracellular structures. Peroxynitrite promotes the release of poly ADP-ribose polymerase 1 (PARP1) and activates the transient receptor potential cation (TRP) channel. PARP1 activation causes  $\text{NAD}^+$  consumption and its consequent depletion, thereby prompting necrotic cell death.

TRP channels are a large family of cation channels that are highly expressed in neurons and glial cells and are involved in various types of perception. The TRPM, TRPV, and TRPC families are thought to be responsible for diabetic neuropathic pain. TRPs induce calcium influx and the subsequent membrane depolarization. The cAMP/PKA pathway stimulates TRPs.

TRPM7 may interact with PLCB1, which in turn activates PKC via diacylglycerol formation. PKC phosphorylates and activates the NADPH oxidase, which catalyzes the production of superoxide radicals.

In neurons the steady-state production of ATP is necessary for ion homeostasis and impulse conduction.  $\text{Na}^+/\text{K}^+$  ATPase restores the initial concentration of ions. Diabetes-related ATP depletion results in the accumulation of intracellular Na, and it disturbs the electrochemical gradients of Na and K ions ([Ang et al., 2018](#); [Aslam et al., 2014](#); [Fischer and Waxman, 2010](#); [Kumar et al., 2018](#); [Naziroğlu et al., 2012](#); [Sloan et al., 2018](#)).

## II. Human disease pathways



**FIG. 15** Pathway 3: Pain perception in diabetic neuropathy.

## References

- ICD-10: E11.40. Disorders of plasma-protein metabolism, not elsewhere classified (Endocrine, nutritional and metabolic diseases (E00-E90), E11 Type 2 diabetes mellitus).
- Albers, J.W., Pop-Busui, R., 2014. Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. *Curr. Neurol. Neurosci. Rep.* 14, 473. <https://doi.org/10.1007/s11910-014-0473-5>.
- Ang, L., Cowdin, N., Mizokami-Stout, K., Pop-Busui, R., 2018. Update on the management of diabetic neuropathy. *Diabetes Spectr.* 31, 224–233. <https://doi.org/10.2337/ds18-0036>.
- Aslam, A., Singh, J., Rajbhandari, S., 2014. Pathogenesis of painful diabetic neuropathy. *Pain Res. Treat.* 2014, 412041. <https://doi.org/10.1155/2014/412041>.
- Dewanjee, S., Das, S., Das, A.K., Bhattacharjee, N., Dihingia, A., Dua, T.K., Kalita, J., Manna, P., 2018. Molecular mechanism of diabetic neuropathy and its pharmacotherapeutic targets. *Eur. J. Pharmacol.* 833, 472–523. <https://doi.org/10.1016/j.ejphar.2018.06.034>.
- Edwards, J.L., Vincent, A.M., Cheng, H.T., Feldman, E.L., 2008. Diabetic neuropathy: mechanisms to management. *Pharmacol. Ther.* 120, 1–34. <https://doi.org/10.1016/j.pharmthera.2008.05.005>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Figueroa-Romero, C., Sadidi, M., Feldman, E.L., 2008. Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. *Rev. Endocr. Metab. Disord.* 9, 301–314. <https://doi.org/10.1007/s11154-008-9104-2>.
- Fischer, T.Z., Waxman, S.G., 2010. Neuropathic pain in diabetes—evidence for a central mechanism. *Nat. Rev. Neurol.* 6, 462–466. <https://doi.org/10.1038/nrneurol.2010.90>.
- Kumar, A., Kaur, H., Singh, A., 2018. Neuropathic pain models caused by damage to central or peripheral nervous system. *Pharmacol. Rep.* 70, 206–216. <https://doi.org/10.1016/j.pharep.2017.09.009>.
- Naziroğlu, M., Dikici, D.M., Dursun, S., 2012. Role of oxidative stress and  $\text{Ca}^{2+}$  signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels. *Neurochem. Res.* 37, 2065–2075. <https://doi.org/10.1007/s11064-012-0850-x>.
- O'Brien, P.D., Hinder, L.M., Sakowski, S.A., Feldman, E.L., 2014. ER stress in diabetic peripheral neuropathy: a new therapeutic target. *Antioxid. Redox Signal.* 21, 621–633. <https://doi.org/10.1089/ars.2013.5807>.
- Shakher, J., Stevens, M.J., 2011. Update on the management of diabetic polyneuropathies. *Diabetes Metab. Syndr. Obes. Targets Ther.* 4, 289–305. <https://doi.org/10.2147/DMSO.S11324>.
- Sifuentes-Franco, S., Pacheco-Moisés, F.P., Rodríguez-Carrizalez, A.D., Miranda-Díaz, A.G., 2017. The role of oxidative stress, mitochondrial function, and autophagy in diabetic polyneuropathy. *J. Diabetes Res.* 2017, 1673081. <https://doi.org/10.1155/2017/1673081>.
- Sloan, G., Shillo, P., Selvarajah, D., Wu, J., Wilkinson, I.D., Tracey, I., Anand, P., Tesfaye, S., 2018. A new look at painful diabetic neuropathy. *Diabetes Res. Clin. Pract.* 144, 177–191. <https://doi.org/10.1016/j.diabres.2018.08.020>.
- Vincent, A.M., Callaghan, B.C., Smith, A.L., Feldman, E.L., 2011. Diabetic neuropathy: cellular mechanisms as therapeutic targets. *Nat. Rev. Neurol.* 7, 573–583. [https://doi.org/10.1038/nrnueurol.2011.137](https://doi.org/10.1038/nrneurol.2011.137).
- Volpe, C.M.O., Villar-Delfino, P.H., Dos Anjos, P.M.F., Nogueira-Machado, J.A., 2018. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis.* 9, 119. <https://doi.org/10.1038/s41419-017-0135-z>.
- Yagihashi, S., Mizukami, H., Sugimoto, K., 2011. Mechanism of diabetic neuropathy: where are we now and where to go? *J. Diabetes Investigig.* 2, 18–32. <https://doi.org/10.1111/j.2040-1124.2010.00070.x>.

## CHAPTER

## 4.4

## Hypothyroidism

Hypothyroidism, regardless of what caused it, has consistent signs of slow metabolism. Untreated hypothyroidism manifests itself in fatigue, weakness, constipation, weight gain, cold intolerance, dry and thick skin, muscle weakness, slow speech, and poor memory.

Hypothyroidism is a disorder caused by the inadequate secretion of thyroid hormone. (*Ferri and Ferri, 2018*).

The thyroid gland produces iodine-containing hormones that play important roles in growth regulation, brain and bone development, cardiovascular function, the control of carbohydrate, lipid and protein metabolism, and oxygen consumption.

The lack of thyroid hormones due to thyroid gland hypofunction is called primary hypothyroidism. Secondary hypothyroidism arises from the inadequate production of thyroid-stimulating hormone by the pituitary gland. A deficiency of thyrotropin-releasing hormone that is released by the hypothalamus causes tertiary hypothyroidism.

Hypothyroidism is a widespread disorder, and primary hypothyroidism comprises the majority of observed cases. In general, hypothyroidism is more common in women than men, and its rate increases with age.

Iodine deficiency in iodine-deficient geographic regions worldwide and Hashimoto's autoimmune thyroiditis in the iodine-sufficient regions are the most common causes of primary hypothyroidism in adults. Other common causes of primary hypothyroidism include drugs, for example, amiodarone and lithium; thyroid therapy with radioactive iodine; or thyroid reduction surgery common in the treatment of Grave's disease (toxic diffuse goiter).

Central hypothyroidism (secondary and tertiary hypothyroidism) is linked to hypothalamic-pituitary discoordination of thyroid hormone production, defective hypothalamus or pituitary development and their dysfunction due to metastatic cancers, infiltrative lesions, infections, stroke, and traumatic brain injury.

Sometimes, hypothyroidism may be caused by tissue resistance to thyroid hormones (Chaker et al., 2017; Diaz and Lipman Diaz, 2014; Patil and Jialal, 2018; Rizzo et al., 2017).

Congenital hypothyroidism occurs in newborns when the thyroid gland is either absent or significantly reduced in size or it cannot function. Thyroid hormone deficiency has more severe consequences when it occurs in babies rather than adults. Hypothyroidism in children leads to the retardation of physical and mental development, cretinism, and the disruption of metabolism and thermoregulation. The most common causes of congenital hypothyroidism are a shortage of iodine in the maternal diet or untreated maternal hypothyroidism during pregnancy. Inheritance accounts for about 20% of the cases of congenital hypothyroidism. For example, mutations in the *PAX8* and *TSHR* genes disrupt normal thyroid gland development. Mutations in the *DUOX2*, *SLC5A5*, *TG*, and *TPO* genes disrupt thyroid hormone synthesis (Genetics Home Reference, <https://ghr.nlm.nih.gov>) (Persani et al., 2018).

Suspected types of disease associated with hypothyroidism can be diagnosed by measuring thyroid hormone levels. Low levels of free thyroxine (FT4) that is released by the thyroid gland and high levels of pituitary thyrotropin (TSH) usually indicate the presence of primary hypothyroidism, while both low levels of T4 and TSH indicate the presence of secondary hypothyroidism (hypopituitarism) (Ferri and Ferri, 2018).

Iodine and hormones released by the hypothalamus and the pituitary gland are primary regulators of thyroid gland function. However, other triggers also may affect the function of thyroid follicular cells leading to decreased secretion of the thyroid hormones, namely, triiodothyronine (T3) and thyroxine (T4):

**Pathway 1.** Decreased secretion of thyroid hormones. (Fig. 16)

Thyroid hormones act through their nuclear receptors that are ubiquitously expressed in human tissues. The effects of thyroid hormones are cell-specific, and they regulate the metabolism in and other functions of tissues and organs.

**Pathway 2.** Effects of thyroid hormone deficiency.

General pathways of thyroid hormone signaling (Fig. 17);

Cell-specific effects of thyroid hormone action (Fig. 18);

Levothyroxine systemic effects (Fig. 19).

## Key cellular contributors and processes

Cardiomyocyte

Cell

Cardiomyocytes are the principal muscular cells that make up the heart, and they are responsible for generating contractile force.

Bone remodeling

Process

Bone remodeling is a dynamic process that maintains bone strength and ion homeostasis by replacing discrete parts of old bone with newly synthesized bone matrix. Bone resorption is performed by large immune cells called osteoclasts, while osteoblasts, a type of specialized connective tissue-related cell, are responsible for making new bone. Bone remodeling is impaired in osteopetrosis due to inadequate osteoclast function and the consequent impairment of bone resorption.

Homeostasis

Process

Homeostasis is self-regulation or the ability of a system to maintain a stable equilibrium and constancy of its internal state through coordinated reactions.

## Pathway 1

### Decreased secretion of thyroid hormones (Fig. 16)

#### Incoming signals

The hypothalamus secretes thyrotropin-releasing hormone (TRH) that in turn induces the secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary gland.

TSH stimulates the thyroid gland to produce the thyrotrophic hormones thyroxine (T4) and to a lesser extent triiodothyronine (T3). T4 synthesis relies on the availability of sufficient levels of iodine. T4 has low biological activity, and it is converted into the more active T3 by the selenium-dependent deiodinase in peripheral tissues. Therefore, iodine and selenium are essential for the production of thyroid hormones.

Circulating levels of T3 and T4 provide negative feedback regulation of TRH and TSH production. Both thyroid hormones, through their nuclear receptors (THRB and THRA), directly inhibit the synthesis and release of TSH in pituitary cells. Also, T3 inhibits the preprocessing of the TRH protein in the hypothalamus.

The destruction of thyroid follicles by autoreactive CD4 T cells in Hashimoto's thyroiditis is considered the most common cause of *dysfunctional secretion of thyroid hormones*. Overall the molecular mechanisms leading to decreased thyroid hormone production are not well understood.

#### Outcome effects

Insufficient triiodothyronine (T3) and thyroxine (T4) production impacts many cell types, slowing down their cell cycle progression, overall metabolism, and cell-type specific functions.

#### Signaling

##### ***Thyrotropin-releasing hormone***

Thyrotropin-releasing hormone (TRH, thyroliberin) is a tripeptide (pyro-Glu-His-Pro-NH<sub>2</sub>) hormone. TRH is mainly produced in the hypothalamic neurons of the hypothalamus paraventricular nucleus (PVN) as a larger inactive precursor, and it undergoes posttranslational modifications by the action of either the prohormone convertases 1/2/3 (PCSK1/2/3) or furin.

The intermediate products of these enzymatic cleavages are subjected to additional modifications by the carboxypeptidase E (CPE) and peptidyl-glycine alpha-amidating monooxygenase enzymes.

T<sub>3</sub> negatively regulates PCSK1/2 expression. The thyroid hormone receptor beta-2 (THRB) and thyroid hormone transporter SLC16A2 are responsible for this T<sub>3</sub>-mediated feedback response. The posttranslational regulation of pro-TRH processing may play an important role in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis and the pathophysiology of hypothyroidism.

Various studies have shown a downregulation of the HPT axis during fasting that supposedly serves as a homeostatic mechanism to reduce catabolism. Low serum levels of leptin protein (LEP) during fasting contribute to the downregulation of TRH neurons in the PVN by having both direct and indirect effects on the hypothalamus. LEP can act directly on TRH neurons by binding to the long isoforms of the leptin receptor (LEPR), which leads to the activation of STAT3 signaling. Also, LEP can stimulate TRH expression indirectly by increasing the levels of released pro-opiomelanocortin (POMC), the precursor of alpha-melanotropin (alpha-MSH). Alpha-MSH enhances TRH synthesis through melanocortin 4 receptor (MC4R) signaling and the related CREB activation.

On the contrary, neuropeptide Y (NPY) suppresses the posttranslational processing of POMC, and it is inhibited by LEP. The action of NPY on TRH neurons is mediated by the activation of signaling by the neuropeptide Y receptors Y1/5 (NPY1R), which decreases CREB levels via the G protein GNAI1 cascade.

The other hormones and catecholamines that can regulate TRH neurons including dopamine, serotonin, histamine, somatostatin, vasoactive intestinal polypeptide, gamma-aminobutyric acid, cytokines, and endogenous opioid peptides such as beta-endorphin, enkephalin, and dynorphin (Lechan and Fekete, 2006; Nillni, 2010; Ortiga-Carvalho et al., 2016).

Mature TRH is transported by the portal vascular system to the anterior pituitary gland where it acts on thyrotrophic and lactotrophic cells to promote the secretion of TSH and prolactin, respectively (Perello et al., 2006).

### **Thyroid stimulating hormone**

Thyroid stimulating hormone (TSH, thyrotropin) is a heterodimer consisting of alpha and beta subunits tightly, but noncovalently, bound to each other. Several inherited TSH beta gene mutations are responsible for familial central hypothyroidism.

TRH is the major positive regulator of TSH beta gene expression and acts mainly by activating the thyrotropin-releasing hormone receptor (TRHR). TRHR is a G protein-coupled receptor located in pituitary thyrotropes that activate phospholipase C, mobilizes Ca<sup>2+</sup>, and activates protein kinase C to stimulate TSH expression (Joseph-Bravo et al., 2016).

In humans, TSH is secreted in low-amplitude pulses with a frequency of 1 pulse every 1–2 h. These low-amplitude pulses, together with the long half-life of TSH (approximately 50 min), result in little variation in

the levels of circulating TSH over time. However, daily TSH secretion follows a characteristic circadian pattern with a peak between 11 p.m. and 4 a.m., followed by low, stable serum levels between 11 a.m. and 11 p.m. The mechanism that generates these TSH pulses is poorly understood.

Dopamine reduces TSH secretion. Other hormones and factors such as cold stress are also implicated in the complex regulation of TSH beta gene expression.

Epinephrine and norepinephrine stimulate TSH secretion in response to adrenergic activation due to acute cold exposure and also in a tonic manner (Fliers et al., 2014; Ortiga-Carvalho et al., 2016).

In hypothyroidism, reduced levels of circulating thyroid hormones lead to high levels of the TSH secretion. Through both of their nuclear receptors (THRB and THRA), thyroid hormones directly inhibit the synthesis and release of TSH by the pituitary. Thyroid hormones can indirectly regulate TSH levels via their effects on TRH synthesis in hypophysiotropic neurons and on LEP synthesis in adipocytes (Mariotti and Beck-Peccoz, 2000).

### **TSHR signaling and thyroid follicle cells**

TSH stimulates the thyroid gland to secrete hormones. The figure displays several central well-known signaling pathways activated by TSH receptor (TSHR) including those acting through G proteins that lead to cAMP synthesis, those resulting in intracellular calcium release and the activation of JAK1/2/STAT3 signaling. TSHR signaling promotes the expression of TPO and thyroglobulin (TG) in thyroid cells.

For the synthesis of thyroid hormones, TPO catalyzes the iodization of tyrosine residues in thyroglobulin to form a complex of TG with both monoiodotyrosine (MIT) and diiodotyrosine (DIT). For this reaction to occur, iodine from food is taken up by the sodium iodide transporters on thyroid follicle cells. Then, DITs are coupled by TPO to create thyroglobulin-iodothyronines, which are in turn hydrolyzed by lysosome proteases to form T4 and T3 that are released into the blood (Rousset et al., 2000).

Mutations in the genes involved with TSHR signaling may lead to the downregulation of thyroid hormone synthesis and a diminished rate of thyroid cell proliferation. For example, defects in TPO alter its ability to couple hydrogen peroxide and iodine to TG on the membranes of thyroid follicle cells. Mutations in SLC26A4 (pendrin), which acts as a chloride-iodide transporter in the thyroid gland and the inner ear, causes Pendred syndrome with its characteristic hearing loss and thyroid goiter (Hannoush and Weiss, 2017; Rastogi and LaFranchi, 2010).

Insensitivity of thyroid cells to TSH, the so-called TSH resistance, can also cause hypothyroidism. TSH resistance is the condition that occurs when high levels of serum TSH are accompanied by normal or reduced levels of serum thyroid hormones. Depending on the degree of TSH

resistance, disease manifestations may be extremely variable, ranging from severe hypothyroidism to mild elevations of TSH levels in the absence of hypothyroidism. Defects in any step along the TSHR signaling pathway in thyroid cells may cause TSH resistance. For example, 23 loss-of-function mutations in TSHR have been documented, and all of them are associated with TSH resistance ([Cassio et al., 2013](#)).

### ***Hashimoto's thyroiditis***

Hashimoto's thyroiditis (HT) results from a complex combination of genetic, environmental, and endogenous factors that interplay to initiate autoimmune reactions against thyroid cells, thereby resulting in hypothyroidism. Thyroid peroxidase (TPO), precursors of thyroid hormones, and thyroglobulin become autoantigens in HT. HT is considered to be a Th1 cell-mediated disease, and it is associated with the infiltration of the thyroid gland by lymphoid cells and resulting destruction of thyroid follicles. The active phase of Hashimoto's thyroiditis is characterized by the apparently uncontrolled production of autoreactive CD4+ T cells, CD8+ cytotoxic T cells, and immunoglobulin G autoantibodies ([Caturegli et al., 2007](#)).

## II. Human disease pathways

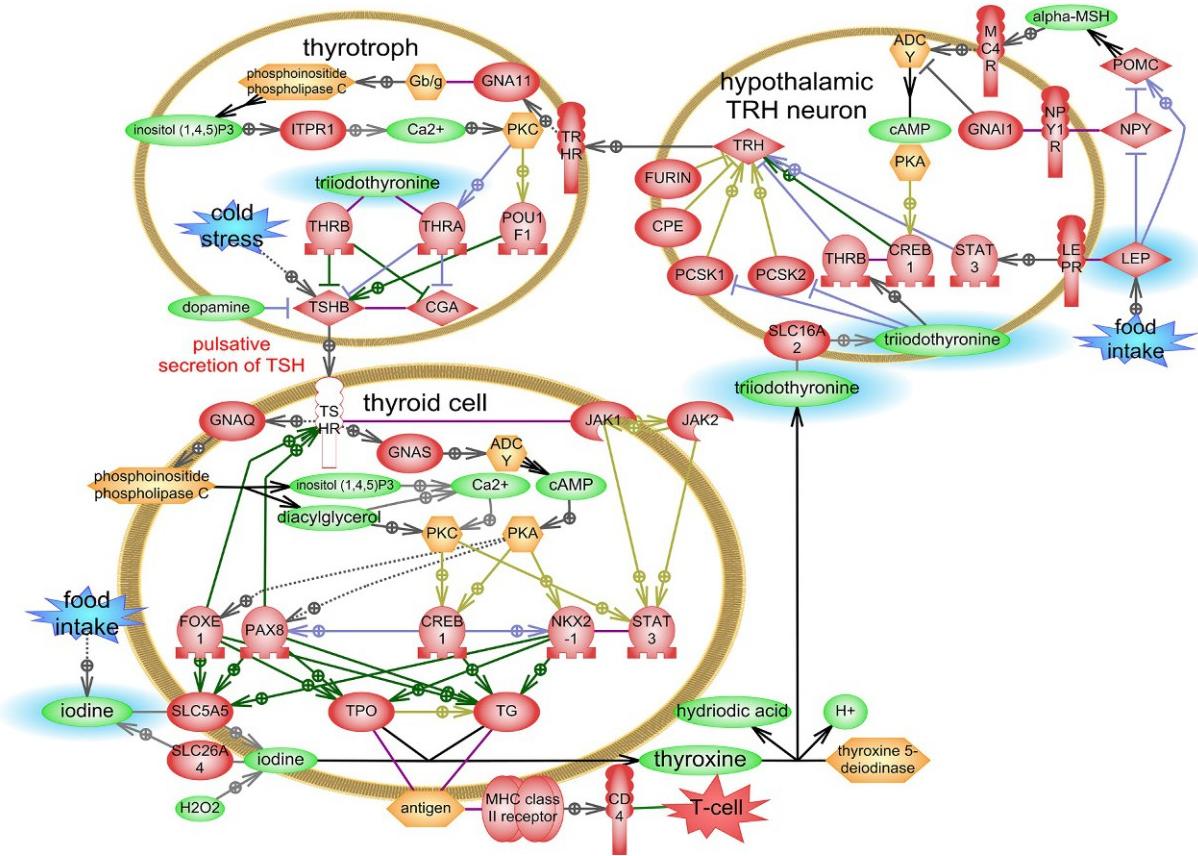


FIG. 16 Pathway 1: Decreased secretion of thyroid hormones.

## Pathway 2

### Cellular effects of thyroid hormone deficiency

#### Incoming signals

There are genomic and nongenomic molecular mechanisms of thyroid hormone action on target cells.

In the case of genomic regulation, thyroid hormone nuclear receptors (THR) mediate the biological activities of triiodothyronine (T3) via the highly gene-specific regulation of transcription. Two THR genes (THRA and THRB) encode four T3 receptor isoforms. THR isoforms display tissue-dependent and time-dependent expression. THRA is the predominant THR isoform in the brain, heart, and bones, while THRB is the major isoform in the kidney and thyroid.

The nongenomic actions of thyroid hormones may be initiated at the plasma membrane, in the cytoplasm, or at intracellular organelles such as the mitochondria, and they do not primarily involve thyroid hormone nuclear receptors.

#### Outcome effects

THR activates many specific cellular processes. The main target organ for thyroid hormones is the heart, and noticeable changes in cardiac function occur in patients with hypo- and hyperthyroidism. Also, thyroid hormones play a crucial role in cholesterol and lipid metabolism in the liver, in skeletal growth, and in the maintenance of bone mass.

#### Signaling

##### ***General pathways of thyroid hormone signaling (Fig. 17)***

Albumin (ALB), transthyretin (TTR), and thyroxine-binding globulin (SERPINA7) stabilize thyroid hormones during circulation in the serum. Triiodothyronine (T3) and thyroxine (T4) penetrate target cells with the help of the solute carrier organic anion transporters family members 1C1 and 6A2 (SLCO1C1 and SLC16A2).

In the cell the prohormone T4 is converted into active T3 by the deiodinases types 1/2 (DIO1 or DIO2). Deiodinase type 3 (DIO3) inactivates T3 and T4 (Brent, 2012).

The binding of T3 to nuclear THRs mediates genomic T3 effects, which results in increased transcription levels of T3-responsive genes. THRs compete with the retinoic acid (RA) nuclear receptor, the vitamin D receptor (VDR), and the peroxisome proliferator-activated receptors (PPAR) for binding and for heterodimerization of the retinoid-X receptor (RXR).

The binding of T3 causes the dissociation of corepressors and the subsequent binding of coactivators to THRs that in turn increase histone acetylation and recruit RNA polymerase II that lead to chromatin remodeling and the activation of the target genes.

For example, ligand-bound heterodimers of RXR-THR interact with the nuclear receptor coactivators NCOA1/2/3, which in turn bind to several chromatin-modifying proteins including histone acetyltransferase CREBBP, coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine N-methyl transferase 1 (PRMT1).

MED1 (TRAP220) is a key component of the mediator complex that forms the link between transcriptional activators and RNA polymerase II. The association of TRH with T3 induces MED1 to recruit other subunits of mediator complex, thereby promoting target gene transcription.

Unliganded heterodimers of RXR-THR bind to thyroid response elements in the genome and repress transcription via corepressors including the nuclear receptor corepressor 1 (NCOR1) and the silencing mediator for retinoid and thyroid hormone receptors NCOR2 (or SMRT). Corepressors require the enzymatic activity of histone deacetylase 3 (HDAC3) that represses transcription ([Liu et al., 2006](#); [Park et al., 2005](#); [Vella and Hollenberg, 2017](#)).

The nongenomic actions of thyroid hormones require proteins other than the THRs. Although T3 can bind to THRB in the cytoplasm, cytoplasmic THRB activation is linked to the shuttling of THRs from the cytoplasm to the nucleus, and it does not stimulate a rapid cellular response. However, thyroid hormones stimulate the release of calcium and canonical cell proliferative cascades such as the mitogen-activated protein kinases (2MAP2K1 and MAP2K2) and phosphatidylinositol 3-kinase/AKT1 through membrane vitronectin receptor signaling.

Also, thyroid hormones regulate the function of plasma membrane ion pumps like  $\text{Ca}^{2+}$ -ATPase and  $\text{Na}/\text{K}$ -ATPase via both genomic and nongenomic cascades. Decreased  $\text{Ca}^{2+}$ -ATPase activity in several human cell types has been shown in patients with hypothyroidism ([Cheng et al., 2010](#); [Davis et al., 2008, 2016](#)).

### **Cell-specific effects of thyroid hormone action (Fig. 18)**

T3 plays a principal role in skeletal homeostasis and bone remodeling. Bone remodeling is the process of local resorption and new bone formation to maintain skeletal balance. Linear growth and bone maturation are tightly regulated by a local feedback loops involving Indian hedgehog (IHH) and parathyroid hormone-like proteins (PTHLH), thyroid hormones, glucocorticoids, sex steroids, various cytokines, growth factors (WNT, BMPs, FGFs, vascular endothelial growth factors), and other inducers that act in paracrine and autocrine manners.

T<sub>3</sub> mediates bone resorption through the activation of osteoclasts and also by inhibiting cellular proliferation and inducing chondrocyte differentiation via complex pathways. For example, T<sub>3</sub> potentiates osteoblast responses to parathyroid hormone by modulating the expression of the parathyroid hormone 1 receptor (PTH1R). In adults, hypothyroidism leads to an imbalance between bone resorption and bone formation, resulting in a slowdown of bone remodeling that leads to bone structural damage.

Furthermore, T<sub>3</sub> stimulates the expression of genes that regulate cartilage matrix synthesis and mineralization, including the matrix proteoglycans that degrade collagen. However, details of the mechanism of T<sub>3</sub> activity in bones are not well known. The expression of THRA and THRB has been described in growth plate chondrocytes, osteoblasts, and the stromal cells of bone marrow, and it may mediate their development through genomic and nongenomic actions (Bassett and Williams, 2016; Wojcicka et al., 2013).

T<sub>3</sub> plays a central role in regulating lipid metabolism (lipogenesis and lipolysis). T<sub>3</sub> influences different key processes in lipid metabolism including the uptake of cholesterol from circulating blood by LDLR, ACACA-mediated cholesterol biosynthesis, and the CYP7A1-mediated synthesis of bile acids in which cholesterol is used as a substrate.

Thyroid hormones induce de novo lipogenesis via the transcription of several lipogenic genes such as acetyl-CoA carboxylase alpha (ACACA), fatty acid synthase (FASN), and thyroid hormone responsive protein (THRSP). In addition, thyroid hormone indirectly controls hepatic lipogenesis by regulating other transcription factors such as the sterol regulatory element-binding protein 1C (SREBF1) or the liver X receptor (NR1H3).

T<sub>3</sub> stimulates lipolysis in adipocytes to generate free fatty acids (FFAs) that can enter hepatocytes and participate in metabolic reactions. FFAs are typically esterified to form triacylglycerol and are subsequently packaged into VLDL for export or to be stored as intracellular lipid droplets. Triacylglycerol stored as lipid droplets can also be hydrolyzed back into FFAs via the action of classic lipases. All of these proteins are regulated at the transcriptional level by thyroid hormone receptor activation. Thus, a deficiency of thyroid hormones changes the metabolism of cholesterol and fatty acids in the liver, and it disrupts both lipogenesis and lipolysis (Damiano et al., 2017; Sinha et al., 2018).

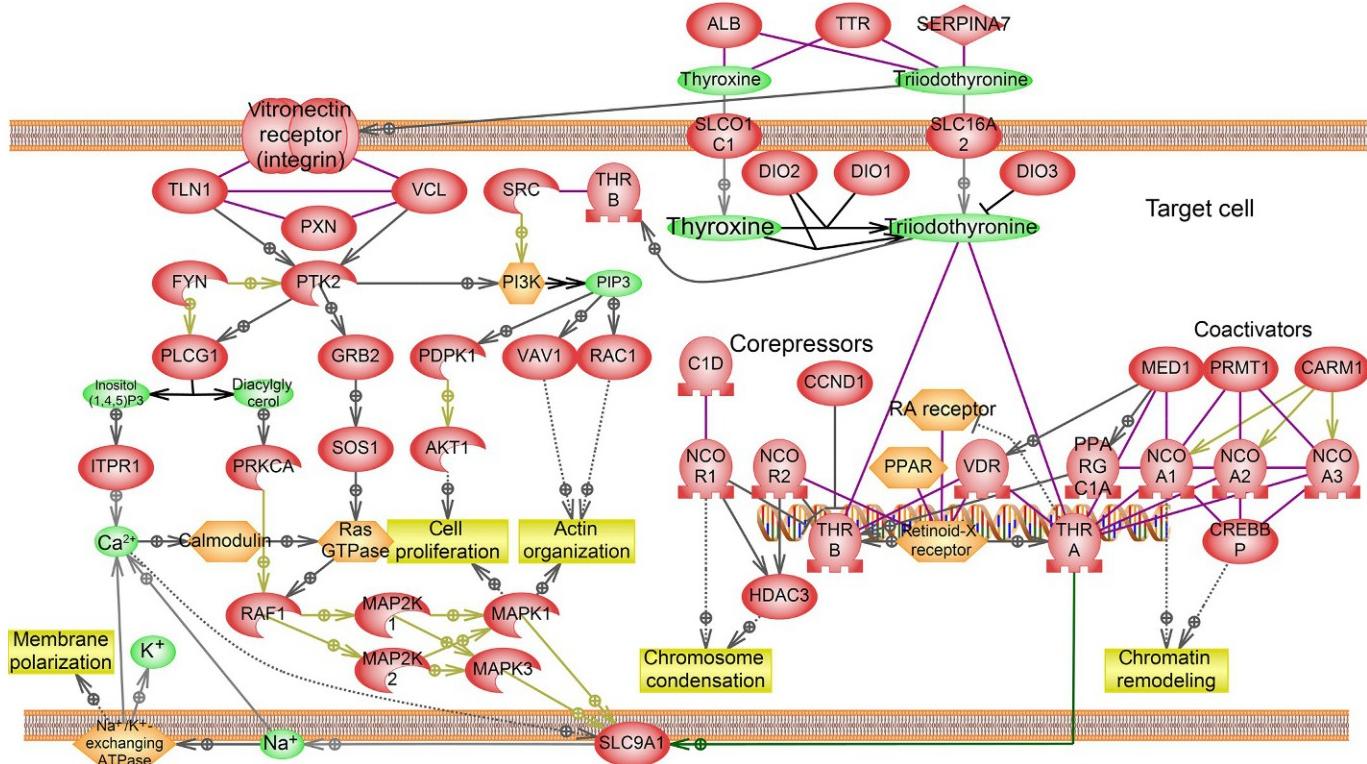
T<sub>3</sub> acts in cardiomyocytes, regulating myocardial contractility and systolic function through THRA, the predominant isoform of THR in the heart. T<sub>3</sub> activates the expression of genes that encode sodium/potassium-transporting ATPases, myosin heavy chain alpha (MYH6), and the endoplasmic reticulum calcium ATPase 2 (ATP2A2), and it negatively regulates the transcription of myosin heavy chain beta (MYH7) and phospholamban (PLN).

The myosin heavy chains are significant components of the cardiomyocyte contractile apparatus. ATP2A2 and its inhibitor PLN regulate  $\text{Ca}^{2+}$  reuptake and release from the cardiac endoplasmic reticulum that in turn regulate diastolic heart muscle relaxation. T<sub>3</sub> increases ATP2A2 levels and decreases PLN levels to promote the reuptake of  $\text{Ca}^{2+}$  during diastole, leading to improved ventricular relaxation.

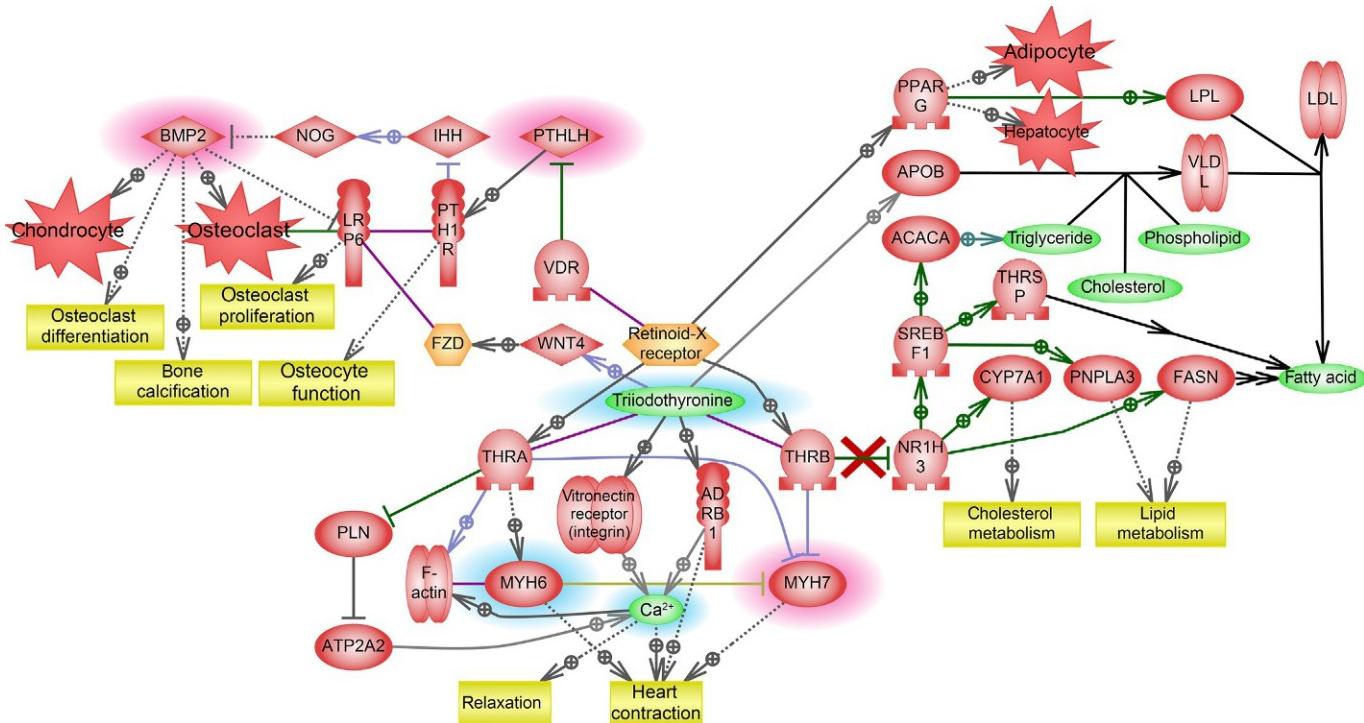
T<sub>3</sub> increases the force of heart contraction by stimulating the expression of the beta 1adrenergic receptor (ADRB1). Also, T<sub>3</sub> has a positive chronotropic effect (increase in the frequency of heart contractions) through both its genomic and nongenomic effects on the activation of adrenergic receptors and ion channels. Bradycardia (reduced heart rate/slow heart rate), diastolic hypertension, and cardiomegaly (increase in heart sizes) may develop as a result of hypothyroidism ([Jabbar et al., 2017](#); [Kahaly and Dillmann, 2005](#); [Udovcic et al., 2017](#)).

Hypothyroidism is treated with levothyroxine, a drug that can cause different unwanted side effects related to hyperthyroidism ([Fig. 19](#)).

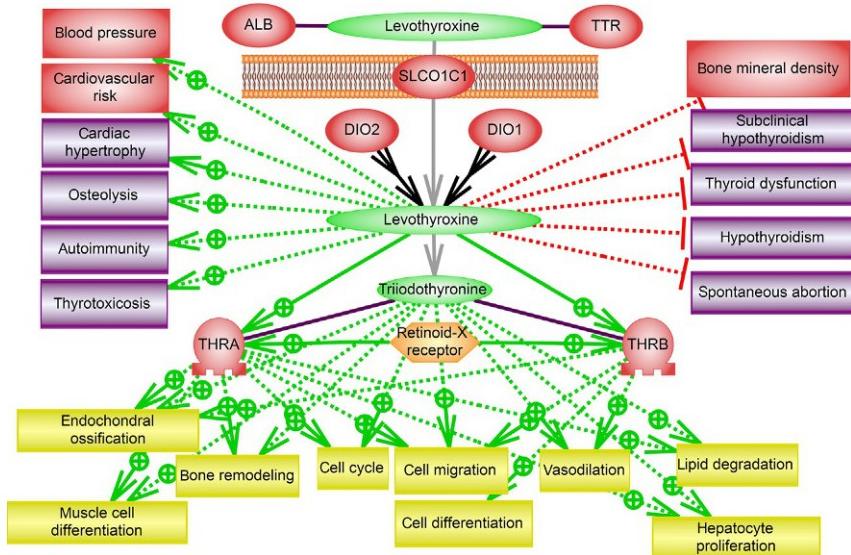
## II. Human disease pathways



**FIG. 17** Pathway 2: General pathways of thyroid hormone signaling.



**FIG. 18** Pathway 2: Effects of thyroid hormone deficiency: cell-specific effects of thyroid hormone action.



**FIG. 19** Pathway 2: Effects of thyroid hormone deficiency: levothyroxine systemic effects.

## References

- Disease numbers #225250, #609893, #275200, #275100, #218700, #614450, #140300 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)). ICD-10: E03-9. (Endocrine, nutritional and metabolic diseases (E00-E90)).
- Bassett, J.H.D., Williams, G.R., 2016. Role of thyroid hormones in skeletal development and bone maintenance. *Endocr. Rev.* 37, 135–187. <https://doi.org/10.1210/er.2015-1106>.
- Brent, G.A., 2012. Mechanisms of thyroid hormone action. *J. Clin. Invest.* 122, 3035–3043. <https://doi.org/10.1172/JCI60047>.
- Cassio, A., Nicoletti, A., Rizzello, A., Zazzetta, E., Bal, M., Baldazzi, L., 2013. Current loss-of-function mutations in the thyrotropin receptor gene: when to investigate, clinical effects, and treatment. *J. Clin. Res. Pediatr. Endocrinol.* 5 (Suppl. 1), 29–39. <https://doi.org/10.4274/jcrpe.864>.
- Caturegli, P., Kimura, H., Rocchi, R., Rose, N.R., 2007. Autoimmune thyroid diseases. *Curr. Opin. Rheumatol.* 19, 44–48. <https://doi.org/10.1097/BOR.0b013e3280113d1a>.
- Chaker, L., Bianco, A.C., Jonklaas, J., Peeters, R.P., 2017. Hypothyroidism. *Lancet* 390, 1550–1562. [https://doi.org/10.1016/S0140-6736\(17\)30703-1](https://doi.org/10.1016/S0140-6736(17)30703-1).
- Cheng, S.-Y., Leonard, J.L., Davis, P.J., 2010. Molecular aspects of thyroid hormone actions. *Endocr. Rev.* 31, 139–170. <https://doi.org/10.1210/er.2009-0007>.
- Damiano, F., Rochira, A., Gnoni, A., Siculella, L., 2017. Action of thyroid hormones, T<sub>3</sub> and T<sub>2</sub>, on hepatic fatty acids: differences in metabolic effects and molecular mechanisms. *Int. J. Mol. Sci.* 18. <https://doi.org/10.3390/ijms18040744>.
- Davis, P.J., Leonard, J.L., Davis, F.B., 2008. Mechanisms of nongenomic actions of thyroid hormone. *Front. Neuroendocrinol.* 29, 211–218. <https://doi.org/10.1016/j.yfrne.2007.09.003>.
- Davis, P.J., Goglia, F., Leonard, J.L., 2016. Nongenomic actions of thyroid hormone. *Nat. Rev. Endocrinol.* 12, 111–121. <https://doi.org/10.1038/nrendo.2015.205>.
- Diaz, A., Lipman Diaz, E.G., 2014. Hypothyroidism. *Pediatr. Rev.* 35, 336–347. quiz 348–349 <https://doi.org/10.1542/pir.35-8-336>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fliers, E., Boelen, A., van Trotsenburg, A.S.P., 2014. Central regulation of the hypothalamo-pituitary-thyroid (HPT) axis: focus on clinical aspects. *Handb. Clin. Neurol.* 124, 127–138. <https://doi.org/10.1016/B978-0-444-59602-4.00009-5>.
- Hannoush, Z.C., Weiss, R.E., 2017. Defects of thyroid hormone synthesis and action. *Endocrinol. Metab. Clin. N. Am.* 46, 375–388. <https://doi.org/10.1016/j.ecl.2017.01.005>.
- Jabbar, A., Pingitore, A., Pearce, S.H.S., Zaman, A., Iervasi, G., Razvi, S., 2017. Thyroid hormones and cardiovascular disease. *Nat. Rev. Cardiol.* 14, 39–55. <https://doi.org/10.1038/nrcardio.2016.174>.
- Joseph-Bravo, P., Jaimes-Hoy, L., Charli, J.-L., 2016. Advances in TRH signaling. *Rev. Endocr. Metab. Disord.* 17, 545–558. <https://doi.org/10.1007/s11154-016-9375-y>.
- Kahaly, G.J., Dillmann, W.H., 2005. Thyroid hormone action in the heart. *Endocr. Rev.* 26, 704–728. <https://doi.org/10.1210/er.2003-0033>.
- Lechan, R.M., Fekete, C., 2006. The TRH neuron: a hypothalamic integrator of energy metabolism. *Prog. Brain Res.* 153, 209–235. [https://doi.org/10.1016/S0079-6123\(06\)53012-2](https://doi.org/10.1016/S0079-6123(06)53012-2).
- Liu, Y., Xia, X., Fondell, J.D., Yen, P.M., 2006. Thyroid hormone-regulated target genes have distinct patterns of coactivator recruitment and histone acetylation. *Mol. Endocrinol.* 20, 483–490. <https://doi.org/10.1210/me.2005-0101>.
- Mariotti, S., Beck-Peccoz, P., 2000. Physiology of the hypothalamic-pituitary-thyroid axis. In: De Groot, L.J., Chrousos, G., Dungan, K., Feingold, K.R., Grossman, A., Hershman, J.M., Koch, C., Korbonits, M., McLachlan, R., New, M., Purnell, J., Rebar, R., Singer, F., Vinik, A. (Eds.), *Endotext*. MDText.com, Inc., South Dartmouth, MA.

- Nillni, E.A., 2010. Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. *Front. Neuroendocrinol.* 31, 134–156. <https://doi.org/10.1016/j.yfrne.2010.01.001>.
- Ortiga-Carvalho, T.M., Chiamolera, M.I., Pazos-Moura, C.C., Wondisford, F.E., 2016. Hypothalamus-pituitary-thyroid axis. *Compr. Physiol.* 6, 1387–1428. <https://doi.org/10.1002/cphy.c150027>.
- Park, S.W., Li, G., Lin, Y.-P., Barrero, M.J., Ge, K., Roeder, R.G., Wei, L.-N., 2005. Thyroid hormone-induced juxtaposition of regulatory elements/factors and chromatin remodeling of Crebp1 dependent on MED1/TRAP220. *Mol. Cell* 19, 643–653. <https://doi.org/10.1016/j.molcel.2005.08.008>.
- Patil, N., Jialal, I., 2018. Hypothyroidism. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Perello, M., Friedman, T., Paez-Espinosa, V., Shen, X., Stuart, R.C., Nillni, E.A., 2006. Thyroid hormones selectively regulate the posttranslational processing of prothyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus. *Endocrinology* 147, 2705–2716. <https://doi.org/10.1210/en.2005-1609>.
- Persani, L., Rurale, G., de Filippis, T., Galazzi, E., Muzza, M., Fugazzola, L., 2018. Genetics and management of congenital hypothyroidism. *Best Pract. Res. Clin. Endocrinol. Metab.* 32, 387–396. <https://doi.org/10.1016/j.beem.2018.05.002>.
- Rastogi, M.V., LaFranchi, S.H., 2010. Congenital hypothyroidism. *Orphanet J. Rare Dis.* 5, 17. <https://doi.org/10.1186/1750-1172-5-17>.
- Rizzo, L.F.L., Mana, D.L., Serra, H.A., 2017. Drug-induced hypothyroidism. *Medicina* 77, 394–404.
- Rousset, B., Dupuy, C., Miot, F., Dumont, J., 2000. Thyroid hormone synthesis and secretion. In: De Groot, L.J., Chrousos, G., Dungan, K., Feingold, K.R., Grossman, A., Hershman, J.M., Koch, C., Korbonits, M., McLachlan, R., New, M., Purnell, J., Rebar, R., Singer, F., Vinik, A. (Eds.), *Endotext*. MDText.com, Inc., South Dartmouth, MA (Chapter 2).
- Sinha, R.A., Singh, B.K., Yen, P.M., 2018. Direct effects of thyroid hormones on hepatic lipid metabolism. *Nat. Rev. Endocrinol.* 14, 259–269. <https://doi.org/10.1038/nrendo.2018.10>.
- Udovcic, M., Pena, R.H., Patham, B., Tabatabai, L., Kansara, A., 2017. Hypothyroidism and the heart. *Methodist DeBakey Cardiovasc. J.* 13, 55–59. <https://doi.org/10.14797/mdcj-13-2-55>.
- Vella, K.R., Hollenberg, A.N., 2017. The actions of thyroid hormone signaling in the nucleus. *Mol. Cell. Endocrinol.* 458, 127–135. <https://doi.org/10.1016/j.mce.2017.03.001>.
- Wojcicka, A., Bassett, J.H.D., Williams, G.R., 2013. Mechanisms of action of thyroid hormones in the skeleton. *Biochim. Biophys. Acta* 1830, 3979–3986. <https://doi.org/10.1016/j.bbagen.2012.05.005>.

## CHAPTER

# 4.5

## Alpha-1 antitrypsin deficiency

Alpha-1-antitrypsin (AAT) deficiency is an autosomal recessive condition caused by mutations in the SERPINA1 (serine proteinase inhibitor A1) gene and is characterized by low levels of circulating AAT protein, which may lead to lung or liver disease with the onset of lung symptoms between the ages of 20 and 50.

Alpha-1-antitrypsin deficiency is a genetic deficiency of the protease inhibitor alpha-1-antitrypsin that results in a predisposition to pulmonary emphysema and hepatic cirrhosis. (*Ferri and Ferri, 2018*).

In people with alpha-1-antitrypsin deficiency, smoking or exposure to tobacco smoke accelerates the appearance of emphysema and damage to the lungs. About 10% of infants with alpha-1-antitrypsin deficiency develop liver disease, which often causes jaundice (yellowing of the skin, mucous membranes, and whites of the eyes). Approximately 15% of adults with alpha-1-antitrypsin deficiency develop liver damage (cirrhosis) due to the formation of scar tissue in the liver. Some individuals with alpha-1-antitrypsin deficiency are misdiagnosed with either asthma or COPD (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

More than 150 allelic variants of the SERPINA1 gene have been reported. The most common and most severe SERPINA1 deficiency is related to the Z (Glu342 to Lys) mutation. This mutation leads to the early development of lung symptoms and to liver disease at any age. Low plasma levels of AAT in this case are caused by the intracellular polymerization and accumulation of the misfolded alpha-1-antitrypsin protein and not by the lack of protein synthesis (Hatipoğlu and Stoller, 2016). Patients with who are homozygous for the Z allele (PIZZ phenotype) of the alpha-1-antitrypsin gene often develop emphysema in the fourth to fifth decades of life due to protease-mediated tissue destruction.

**Pathway 1.** *SERPINA1-associated pulmonary emphysema* (Fig. 20). It is estimated that 2%–3% of patients with the Z-type of alpha-1-antitrypsin deficiency develop liver cirrhosis during childhood. The rest of the patients are at an elevated risk for developing liver function abnormalities.

**Pathway 2.** *SERPINA1-associated liver damage* (Fig. 21).

## Key cellular contributors and processes

Endoplasmic reticulum

Anatomic structure

The endoplasmic reticulum (ER) is a cytoplasmic organelle that forms a continuous network of membrane-enclosed tubules and sacs (cisternae). The ER is involved in protein folding and the transport of newly synthesized proteins to the Golgi apparatus.

Usher syndrome

Disease

Usher syndrome is a rare genetic disorder characterized by hearing loss and gradual vision loss due to retinitis pigmentosa, the progressive degeneration of photoreceptor cells in the retina. The disease is clinically and genetically heterogeneous with at least 15 chromosomal loci assigned to three clinical Usher syndrome types, namely, USH1A-G, USH2A-C, and USH3A.

Hepatic cirrhosis

Disease

Hepatic cirrhosis is a chronic degenerative disease characterized by irreversible replacement of normal liver cells with scar tissue resulting from long-term liver damage.

## Pathway 1

### SERPINA1-associated pulmonary emphysema (Fig. 20)

#### Cause and inductors

Alpha-1-antitrypsin (AAT), encoded by the *SERPINA1* gene, is a protein that protects the body from the antibacterial enzyme neutrophil elastase (ELANE), which is released by white blood cells and can attack healthy tissues, especially the lungs if not tightly controlled by AAT. ELANE digests a wide range of extracellular matrix components including elastin (ELN), collagen type I, fibronectin (FN1), and fibrin. AAT is an inhibitor of ELANE that protects the lungs from protease-mediated tissue destruction.

AAT is synthesized in hepatocytes and monocytes and is passively transported to the lungs by normal circulation.

#### Outcome effects

The active elastase enzyme destroys the elastic fibers of the lungs, thereby reducing the pulmonary elasticity required for normal respiration. This progressive destruction results in chronic obstructive bronchitis and pulmonary fibrosis. Further, neutrophil function and the local immune response are diminished. As result, pulmonary emphysema develops, wherein an abnormal permanent enlargement of the airspaces in the bronchioles is accompanied by the destruction of alveolar walls ([Gooptu et al., 2009](#); [Petrache et al., 2006](#); [Stoller and Aboussouan, 2012](#)).

#### Signaling

Excessive degradation of elastin is central to the pathogenesis of emphysema. Elastin is a core component of lung elastic fibers and extracellular matrix and is responsible for lung expansion during respiration.

The loss of elastic tissue plays a major role in several obstructive airway diseases. In alpha-1-antitrypsin deficiency, the majority of proteases released in lung tissue remains active and slowly destroys lung extracellular matrix components, alveolar structures, and pulmonary blood vessels. Cigarette smoke can increase the activity of those proteases.

Elastic fibers are a major component of the large reservoir of growth factors, such as TGFB1. ELANE releases TGFB1 and FGF2 that in turn leads to fibrosis via the activation of growth factor signaling in the cells of pulmonary organs.

Alpha-1-antitrypsin inhibits CASP3 activity and prevents apoptosis of lung endothelial cells; however, this function is impaired when AAT is deficient ([Lockett et al., 2012](#)).

Also, AAT is thought to suppress certain neutrophil functions such as superoxide production or CXCL8 release. ELANE is also implicated in neutrophil suppression through the cleavage of CXCR1, a cell-surface chemokine receptor. On the other hand the binding of ELANE to alveolar macrophages causes the release of leukotriene B4, which is implicated in neutrophil recruitment.

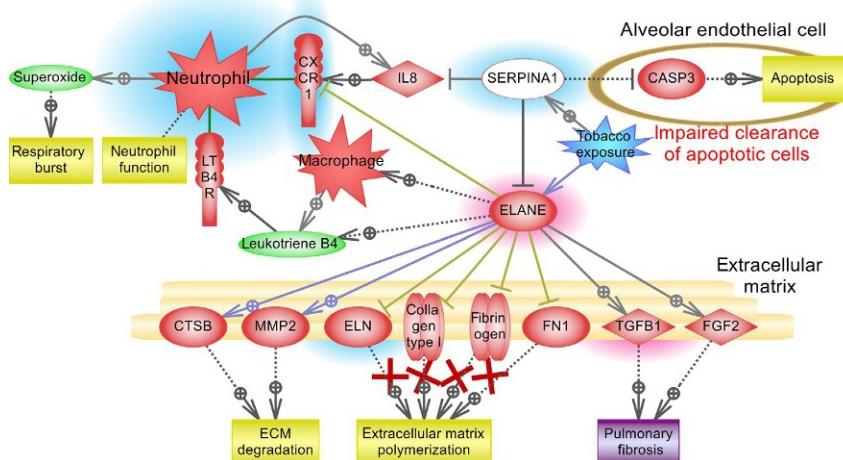


FIG. 20 Pathway 1: SERPINA1-associated pulmonary emphysema.

## Pathway 2

### SERPINA1-associated liver damage (Fig. 21)

#### Cause and inductors

Alpha-1-antitrypsin (AAT) is synthesized by hepatocytes and monocytes, and it is passively transported through normal circulation into the lungs. Approximately 80%–90% of mutant AAT (SERPINA1) protein is retained in the endoplasmic reticulum (ER) of hepatocytes. Retention of the mutant protein within the ER is thought to be the basis for the pathogenesis of hepatic disease.

#### Outcome effects

ER stress causes hepatocyte apoptosis, and it activates expression of inflammatory mediators such as IL-6 and CXCL8. Hepatocyte dysfunction and inflammation can result in hepatic cirrhosis (Lawless et al., 2008; Perlmutter et al., 2007).

#### Signaling

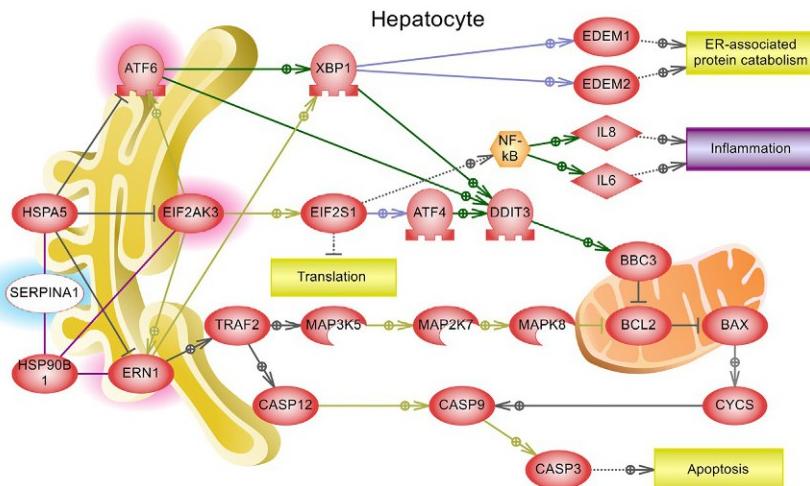
The mutant AAT protein cannot be processed normally after translation. As an unfolded protein, mutant AAT binds HSPA5 (heat shock protein family A member 5), a principal chaperone of unfolded proteins in ER. When bound to AAT, HSPA5 releases the proximal ER stress transducers (ERN1, EIF2AK3, and ATF6).

Under normal conditions, HSPA5 serves as a negative regulator of these transducers, which themselves act at the final step of the unfolded protein response (UPR), namely, the initiation of caspase-dependent cell death.

Endoplasmic reticulum to nucleus signaling 1 (ERN1) serves as a proapoptotic protein by recruiting TNF receptor associated factor 2 (TRAF2) to activate the mitogen-activated protein kinase (MAPK) cascade, mitochondrial regulators of apoptosis, and downstream caspases.

In addition, apoptosis is stimulated by activating transcription factor 6 (ATF6) and X-box binding protein 1 (XBP1), which activate DNA damage inducible transcript 3 (DDIT3). DDIT3 is one of the major ER stress responsive transcription factors that regulate expression of the proapoptotic protein BCL2 binding component 3 (BBC3).

ATF6 activation also leads to the expression of proinflammatory mediators via NF- $\kappa$ B signaling (Jiang et al., 2003) and the activation of ER-associated protein catabolism.



**FIG. 21** Pathway 2: SERPINA1-associated liver damage.

## References

- Disease numbers # 613490 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: E88. Disorders of plasma-protein metabolism, not elsewhere classified (Endocrine, nutritional and metabolic diseases (E00-E90)).
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Gooptu, B., Ekeowa, U.I., Lomas, D.A., 2009. Mechanisms of emphysema in alpha1-antitrypsin deficiency: molecular and cellular insights. *Eur. Respir. J.* 34, 475–488. <https://doi.org/10.1183/09031936.00096508>.
- Hatipoğlu, U., Stoller, J.K., 2016.  $\alpha$ 1-Antitrypsin deficiency. *Clin. Chest Med.* 37, 487–504. <https://doi.org/10.1016/j.ccm.2016.04.011>.
- Jiang, H.-Y., Wek, S.A., McGrath, B.C., Scheuner, D., Kaufman, R.J., Cavener, D.R., Wek, R.C., 2003. Phosphorylation of the  $\alpha$  subunit of eukaryotic initiation factor 2 is required for activation of NF- $\kappa$ B in response to diverse cellular stresses. *Mol. Cell. Biol.* 23, 5651–5663. <https://doi.org/10.1128/MCB.23.16.5651-5663.2003>.
- Lawless, M.W., Mankani, A.K., Gray, S.G., Norris, S., 2008. Endoplasmic reticulum stress—a double edged sword for Z alpha-1 antitrypsin deficiency hepatotoxicity. *Int. J. Biochem. Cell Biol.* 40, 1403–1414. <https://doi.org/10.1016/j.biocel.2008.02.008>.
- Lockett, A.D., Van Demark, M., Gu, Y., Schweitzer, K.S., Sigua, N., Kamocki, K., Fijalkowska, I., Garrison, J., Fisher, A.J., Serban, K., Wise, R.A., Flotte, T.R., Mueller, C., Presson, R.G., Petrache, H.I., Tuder, R.M., Petrache, I., 2012. Effect of cigarette smoke exposure and structural modifications on the  $\alpha$ -1 Antitrypsin interaction with caspases. *Mol. Med.* 18, 445–454. <https://doi.org/10.2119/molmed.2011.00207>.
- Perlmutter, D.H., Brodsky, J.L., Balistreri, W.F., Trapnell, B.C., 2007. Molecular pathogenesis of alpha-1-antitrypsin deficiency-associated liver disease: a meeting review. *Hepatology* 45, 1313–1323. <https://doi.org/10.1002/hep.21628>.
- Petrache, I., Fijalkowska, I., Medller, T.R., Skirball, J., Cruz, P., Zhen, L., Petrache, H.I., Flotte, T.R., Tuder, R.M., 2006. Alpha-1 antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. *Am. J. Pathol.* 169, 1155–1166.
- Stoller, J.K., Aboussouan, L.S., 2012. A review of  $\alpha$ 1-antitrypsin deficiency. *Am. J. Respir. Crit. Care Med.* 185, 246–259. <https://doi.org/10.1164/rccm.201108-1428CI>.

## CHAPTER

# 4.6

## Cystic fibrosis

Cystic fibrosis is an inherited monogenic disease characterized by progressive damage to the respiratory and digestive systems due to the buildup of abnormally thick mucus.

Cystic fibrosis (CF) is an autosomal recessive disorder characterized by dysfunction of exocrine glands. (*Ferri and Ferri, 2018*).

In healthy individuals, mucus lubricates and protects the linings of the airways, digestive system, reproductive system, and other organs and tissues. The overproduction of mucus causes chronic bacterial infections and inflammation in the lungs and a blockage of intestinal and pancreatic ducts.

Cystic fibrosis is associated with various mutations in the CFTR gene that cause mild to severe forms of the disease. These mutations disrupt CFTR function and affect the transport of chloride ions and water across the plasma membranes of epithelial cells.

Cystic fibrosis was once considered a fatal disease of childhood. However, contemporary approaches to treatment based on our current knowledge allow doctors to better manage the disease and save lives. Nevertheless, cystic fibrosis is a life-shortening disorder. In adults with cystic fibrosis, chronic inflammation in their lungs leads to the formation of scar tissue (fibrosis) and cysts. Problems with digestion can lead to chronic diarrhea and malnutrition. Moreover, reduced insulin production in cystic fibrosis may cause a form of diabetes called cystic fibrosis-related diabetes mellitus. Most men with cystic fibrosis have a congenital bilateral absence of the vas deferens (CBAVD), a condition in which the tubes that carry sperm (the vas deferens) are blocked by mucus during embryonic development leading to their deterioration before birth (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

In addition to nucleotide alterations in the CFTR gene, mutations in the *IFRD1*, *TGFB1*, or *MBL* genes were suggested to perturb these modifiers of cystic fibrosis (*Cordovado et al., 2012*).

The diversity of CFTR mutations in cystic fibrosis determines the penetrance of the disease. Six different classes of mutations in the CFTR gene have been linked to cystic fibrosis (Weiler and Drumm, 2013). Class I mutations affect the biosynthesis of CFTR and include the most severe CF phenotypes in which no protein is synthesized. Class II mutations result in folding defects of the CFTR protein. The class II mutation delta F508 (Phe508del mutation) is the most common genetic alteration in cystic fibrosis and leads to the deletion of phenylalanine at position 508. **Pathway 1.** *CFTR expression and degradation in epithelial cells in cystic fibrosis (class I-II mutations)* (Fig. 22).

Class III and class IV mutations cause reductions in transmembrane Cl<sup>-</sup> conductance. Class III mutations, in a manner similar to class II mutations, usually lead to a classic CF phenotype with pancreatic insufficiency.

**Pathway 2.** *Ion channel CFTR failure in cystic fibrosis airway epithelium (class III-IV mutations)* (Fig. 23).

Class V and VI mutations are associated with a milder form of the disease that results from reduced levels of what is otherwise normal CFTR protein.

## Key cellular contributors and processes

Endoplasmic reticulum–associated protein degradation

Process

ERAD stands for endoplasmic reticulum–associated protein degradation and is a cellular process that targets misfolded proteins for degradation by the cytoplasmic ubiquitin-proteasome system.

Fibrosis

Process

Fibrosis is the development of excessive fibrous connective tissue and the accumulation of extracellular matrix proteins in an organ or tissue. This typically occurs as reparative response to tissue damage. Fibrosis leads to scarring and thickening of the affected tissue, and it disrupts its function.

Mucociliary clearance

Process

Mucociliary clearance (MCC) is one of the major defense mechanisms of the lungs in which mucus and potentially harmful foreign substances contained in it are moved out of the lung. Cilia on the surfaces of airway epithelial cells provide the force necessary to move mucus.

Mucus

Process

Mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may be tethered together at the fluid surface by oligomeric mucin protein complexes.

## Pathway 1

### **CFTR expression and degradation in epithelial cells in cystic fibrosis (class I-II mutations) (Fig. 22)**

#### Incoming signals

CFTR is a cyclic AMP (cAMP)-regulated, ATP-gated, chloride ( $\text{Cl}^-$ ) channel located in the apical membrane of epithelial cells lining the airways, intestines, ducts of the pancreas, and sweat glands. The absence or lack of fully functional CFTR causes cystic fibrosis.

The class I G542X mutation in the CFTR gene that is prevalent in cystic fibrosis (Cordovado et al., 2012) prevents the synthesis of a stable CFTR protein or results in the production of a truncated protein due to the creation of a premature termination codon. The truncated proteins are usually unstable and therefore are recognized by chaperone proteins in the endoplasmic reticulum (ER) and rapidly degraded.

The delta-F508 mutation (class II) in CFTR is the most common known mutation in cystic fibrosis, and it results in a folding defect leading to retention of the protein in the endoplasmic reticulum and its subsequent degradation by proteasomes (Cordovado et al., 2012; Rowntree and Harris, 2003; Weiler and Drumm, 2013).

#### Outcome

Cystic fibrosis patients with class I and class II mutations exhibit a typical phenotype characterized by thick mucus secretions, clogging of the airways, and persistent pulmonary infections leading to pulmonary failure and death.

#### Signaling

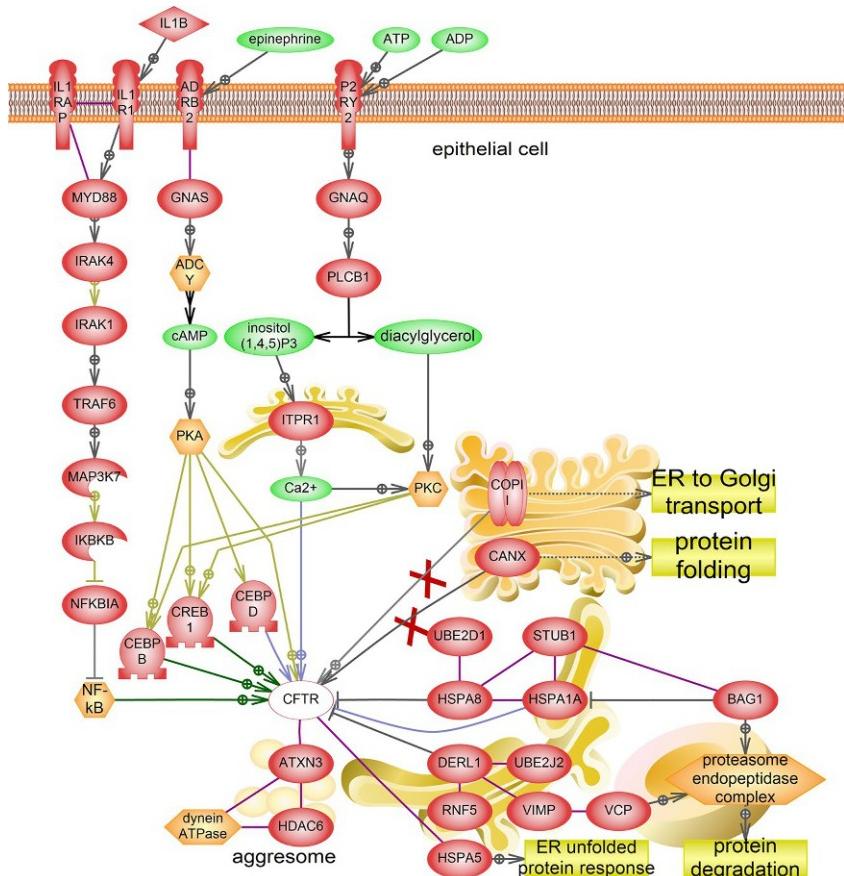
Transcription of the CFTR gene is highly regulated in a time- and cell-specific manner. CFTR is expressed at low levels in postnatal and adult lungs and, conversely, at high levels in fetal lungs. Long distance cis-regulatory sequences influence the gene's promoter and complicate the regulation of *CFTR* transcription (Bergougnoux et al., 2014).

The majority of the data available regarding signal transduction underlying CFTR expression in the lung were obtained from animal models and cell lines and therefore require further research. Several signaling paths initiated by IL-1B, IL-13, IL-4, and the beta-adrenergic receptors (ADRBs) have been shown to be involved in activation of CFTR gene expression. On the other hand, IFNG- or TGFB-related signaling networks are implicated in the inhibition of CFTR expression (Hamacher et al., 2018). Fig. 22

depicts the IL-1R1 and ADRB2 cascades that activate the transcription factors NF- $\kappa$ B and CEBPD, CEBPB, and CREB1 that are associated with CFTR gene expression (Cafferata et al., 2001; Viart et al., 2013).

The cAMP/protein kinase A (PKA) signaling pathway is the major route for the activation of CFTR Cl<sup>-</sup> channel function in airway epithelial cells, although there also exists evidence that CFTR can also be activated by protein kinase C (PKC) and the cGMP-dependent protein kinase. In addition, the purinergic receptor P2Y2 (P2RY2) was suggested to be involved in CFTR activation (Lazarowski and Boucher, 2009; Namkung et al., 2010).

Following CFTR synthesis, the process of its maturation starts in the endoplasmic reticulum (ER); specifically, the newly synthesized CFTR protein is folded and glycosylaminated. CFTR folding is monitored by chaperone proteins associated with the ER-associated protein degradation (ERAD) process. The mutant deltaF508-CFTR protein is recognized as misfolded by the ERAD, shuttled out of the ER, and targeted for proteasomal degradation. Two ubiquitin ligase complexes may be involved in deltaF508-CFTR degradation, namely, the ER membrane-associated ubiquitin ligase complex (DERL1, RNF5, and UBE2J2) and the cytosolic ubiquitin ligase complex (STUB1 and UBE2D1). Mutant CFTR protein is degraded via the 26S proteasome, or it is transported to the aggresome near the microtubule-organizing center. Aggregation of "misfolded" CFTR proteins can be the reason for the unfolded protein response, membrane Cl<sup>-</sup> transport dysfunction, or apoptosis of delta F508-CFTR-expressing cells. A properly folded CFTR is transported to the Golgi apparatus for further maturation before being transferred to the apical cell surface. Calnexin (CANX), coat protein complex II (COP2), and other proteins regulate the maturation and trafficking of the normal CFTR (Ameen et al., 2007; Chanoux and Rubenstein, 2012).



**FIG. 22** Pathway 1: CFTR expression and degradation in epithelial cells in cystic fibrosis (class I-II mutations).

## Pathway 2

### Failure of the ion channel function of CFTR in cystic fibrosis airway epithelium (class III-IV mutations) (Fig. 23)

#### Incoming signals

One of the major functions of CFTR is to regulate the movement of chloride ( $\text{Cl}^-$ ) and sodium ( $\text{Na}^+$ ) ions across epithelial cell membranes, such as the alveolar epithelia located in the lungs.

Class III CFTR mutations disturb the protein's chloride-transporting function. Mutated CFTR does not respond to cAMP stimulation even though it is located in the cell membrane. Class III mutations (as in the case of the previously described two classes) cause a classic phenotype with lung diseases and pancreatic insufficiency.

Class IV mutations also affect  $\text{Cl}^-$  conductance but are mostly associated with milder forms of the disease.

It is worth mentioning that lung disease is the major cause of mortality in cystic fibrosis. Still, it is not completely clear how exactly the disruption of ion transport causes lung disease in cystic fibrosis. The prevailing hypothesis is that disruption of the normal CFTR-mediated negative regulation of epithelial  $\text{Na}^+$  channels (ENaC) leads to excess sodium ( $\text{Na}^+$ ) absorption and therefore dehydration of the airway epithelium.

#### Outputs

As result of class II and class III CFTR mutations, the protective function of the airway surface liquid declines. As a result the dehydrated lubricating layer and the resulting viscous mucus allow bacteria and apoptotic debris to accumulate on the lung epithelium. Compressed by this thick layer of mucus and debris, cilia cannot clear the mucus effectively. Further, trapped pathogens (usually *Staphylococcus aureus* or *Pseudomonas aeruginosa*) activate the NF- $\kappa$ B signaling pathway in adjacent cells, resulting in chronic infection and neutrophilic inflammation. Lung disease results from clogging of the airways due to a mucus buildup, decreased clearance of pathogens, and progressive airway destruction caused by chronic inflammation.

The CFTR protein is anchored to the outer membrane of cells of all exocrine glands in the body (sweat glands, lungs, pancreas, etc.). The dehydration and accumulation of excess mucoproteins in the gastrointestinal tract hinder the removal of pathogens and cellular debris by phagocytes and dendritic cells, thus supporting the development of infection and inflammation in the gut.

## Signaling

### **Airway surface liquid dehydration**

The mechanical clearance of mucus is a major defense mechanism against inhaled microorganisms the airways. Normal mucociliary clearance is mediated by a two-layer liquid system known as the airway surface liquid (ASL). In ASL, the upper phase is the mucus layer that is composed of high-molecular-weight secreted mucins, and the lower layer is known as the periciliary liquid layer (PCL). The interaction between these two layers facilitates effective ciliary function and the consequent airway clearance ([Bustamante-Marin and Ostrowski, 2017](#)).

Adequate hydration of ASL depends on normally regulated levels of sodium ( $\text{Na}^+$ ) absorption by epithelial  $\text{Na}^+$  channels (ENaC), which are responsible for salt and water homeostasis.

The normal CFTR anion channel provides secreted chloride and bicarbonate, and it regulates and, probably, inhibits epithelial sodium channels (ENaC). It is worth noting that the apical (in the case of ENaC) or basolateral (in the case of the  $\text{Na}^+/\text{K}^+$ -ATPase) position of ion channels and the type of alveolar epithelium cells impact water and salt balance in ASL.

In cystic fibrosis, the nonfunctional CFTR causes ENaC to increase  $\text{Na}^+$  absorption by the epithelium, thereby increasing osmotically driven water reabsorption. Notably, a number of water channel proteins or aquaporins (AQPs) are expressed in the lungs including AQP1 in the microvascular endothelia, AQP3 in the large airways, AQP4 in both the large- and small-airway epithelia, and AQP5 in type I alveolar epithelial cells. As a result the volume of airway surface liquid (ASL) decreases and leads to the formation of thick and dehydrated mucus and the subsequent failure to clear of mucus by cilia located on the surface of the epithelium ([Collawn and Matalon, 2014](#); [Hamacher et al., 2018](#); [McCarron et al., 2018](#)).

Another theory proposes that a lack of chloride, iodide, and thiocyanate in the intercellular space due to the CFTR protein channel deficiency leads to an imbalance between the release and elimination of reactive oxygen species (ROS) ([Xu et al., 2009](#)).

### **Mucus production**

Mucus overproduction is a hallmark of cystic fibrosis-related lung disease. The inflammatory cytokines TGF $\alpha$ , IL6, and IL13, free radicals, and pathogen-activated toll-like receptor (TLR) signaling together induce the synthesis of mucins in cystic fibrosis ([Abdullah et al., 2017](#); [Henke et al., 2007](#)). For details on the signaling routes associated with the production of mucins by lung epithelium, see the pathways on Asthma.

### Oxidative stress

The theory of redox imbalance in CF points out that a mutant CFTR channel does not transport antioxidants to counteract oxidative stress in the airway epithelium.

The production of ROS is an important step in many cellular processes. There exist a large number of activators of free radical production by the epithelium, and a release of excess ROS leads to oxidative stress. Chronic infection and inflammation activate macrophages, neutrophils, and eosinophils leading to the production of  $O_2^-$ , which is rapidly converted to  $H_2O_2$  by superoxide dismutase (SOD).  $H_2O_2$  in turn activates NF-kB and mediates other oxidative stress-associated cellular responses.

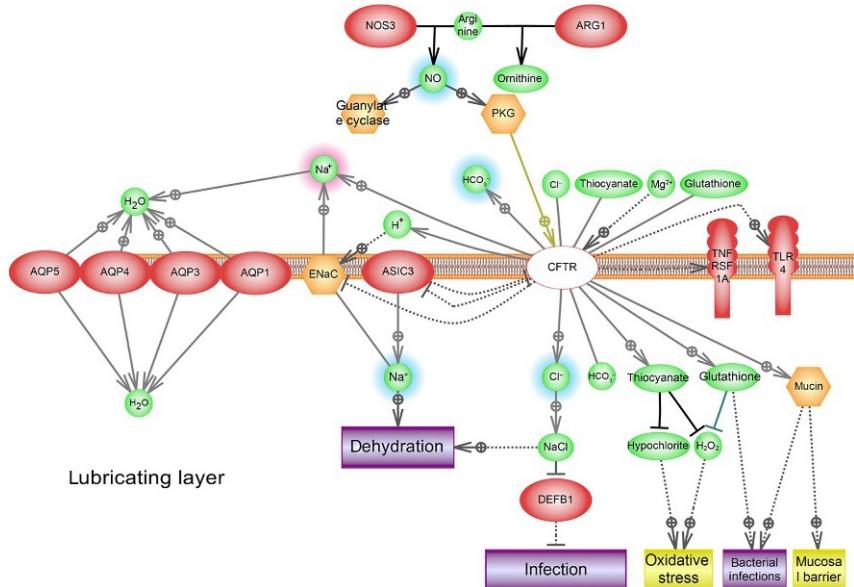
Elevated levels of neutrophil-activating protein 1 (interleukin IL-8, CXCL8) is a marker of cystic fibrosis in the lungs (even in the absence of pathogens). CXCL8 stimulates the accumulation of neutrophils in the airway that increases the production of reactive oxygen species. The mechanisms underlying the increased CXCL8 expression observed in cystic fibrosis are not fully understood (Poghosyan et al., 2016).

In tissues a complex system of redox balance exists to control the effects of oxidants and antioxidants. In healthy epithelial cells, oxidative stress stimulates the synthesis of antioxidants (e.g., glutathione and thiocyanate) that in turn decrease oxidative stress via various mechanisms. For example, glutathione reductase (GSR) uses glutathione as a substrate in the detoxification of peroxides such as  $H_2O_2$  and lipid peroxides.

In the plasma of cystic fibrosis patients, a significant increase in oxidation and decreased levels of antioxidants such as vitamin A, vitamin E, and glutathione have been reported (Ziady and Hansen, 2014). Presumably, in cystic fibrosis, antioxidant levels decrease due to the inability of mutant CFTR proteins to transport glutathione (GSH) and thiocyanate (SCN-) into the airways. Consequently, decreased levels of GSH, SCN, and some enzymes (lactoperoxidase (LPO) and glutathione reductase) disrupt the oxidation of the free radicals that accumulate in the airways (Xu et al., 2009; Zhang et al., 2015; Ziady and Hansen, 2014).

Low levels of exhaled nitric oxide ( $FE_{NO}$ ) serve as a marker of inflammation in cystic fibrosis. Other types of inflammation in the lung are usually accompanied by high NO levels (Dinh-Xuan and Hua-Huy, 2015). NO levels can decrease due to an increased concentration of free radicals or increased activity of ARG1, which itself competes with NOS3 for substrates during NO synthesis (de W. Groot and van der Ent, 2005).

NO also contributes to the normal stimulation of CFTR through the regulation of guanylate cyclase (sGC) (Bellingham and Evans, 2007). The major signaling route for CFTR activation involves adenosine/cAMP/PKA-related signaling. A large number of other mediators may activate CFTR function. Studies on CFTR activators are important for finding new drug targets that can be used in treatment of this chronic and life-shortening disease (Lukowski et al., 2015; Mutlu and Factor, 2008; Watson et al., 2011).



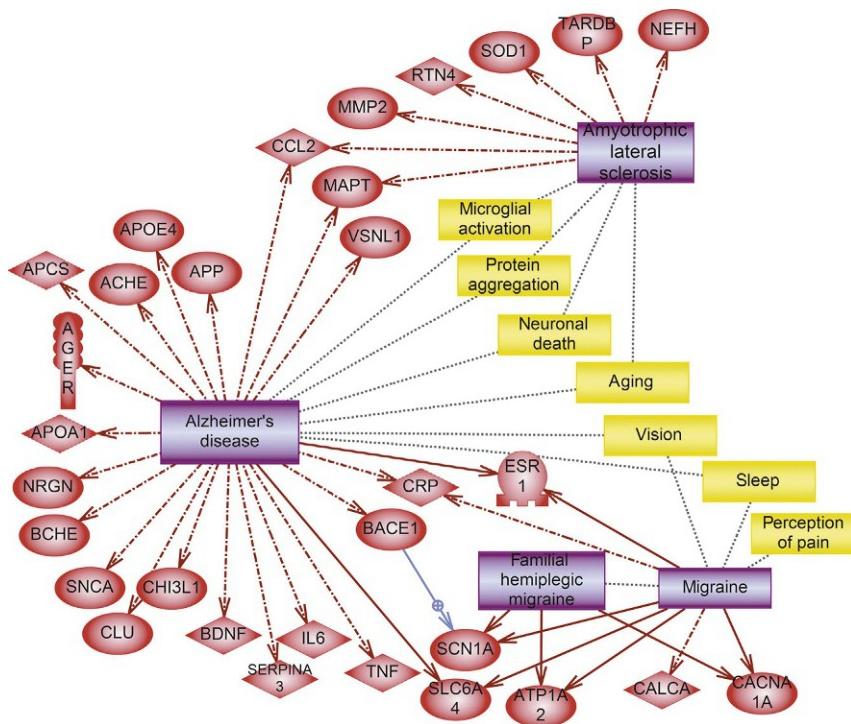
**FIG. 23** Pathway 2: Ion channel CFTR failure in cystic fibrosis airway epithelium (class III-IV mutations).

## References

- Disease numbers # 192600, # 115195, # 600858 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: E84.Endocrine, nutritional and metabolic diseases (E00-E90).
- Abdullah, L.H., Evans, J.R., Wang, T.T., Ford, A.A., Makhov, A.M., Nguyen, K., Coakley, R.D., Griffith, J.D., Davis, C.W., Ballard, S.T., Kesimer, M., 2017. Defective postsecretory maturation of MUC5B mucin in cystic fibrosis airways. *JCI Insight* 2, e89752. <https://doi.org/10.1172/jci.insight.89752>.
- Ameen, N., Silvis, M., Bradbury, N.A., 2007. Endocytic trafficking of CFTR in health and disease. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* 6, 1–14. <https://doi.org/10.1016/j.jcf.2006.09.002>.
- Bellingham, M., Evans, T.J., 2007. The alpha2beta1 isoform of guanylyl cyclase mediates plasma membrane localized nitric oxide signalling. *Cell. Signal.* 19, 2183–2193. <https://doi.org/10.1016/j.cellsig.2007.06.017>.
- Bergognoux, A., Rivals, I., Liquori, A., Raynal, C., Varilh, J., Magalhães, M., Perez, M.-J., Bigi, N., Des Georges, M., Chiron, R., Squalli-Houssaini, A.S., Claustris, M., De Sario, A., 2014. A balance between activating and repressive histone modifications regulates cystic fibrosis transmembrane conductance regulator (CFTR) expression in vivo. *Epigenetics* 9, 1007–1017. <https://doi.org/10.4161/epi.28967>.
- Bustamante-Marin, X.M., Ostrowski, L.E., 2017. Cilia and mucociliary clearance. *Cold Spring Harb. Perspect. Biol.* 9. <https://doi.org/10.1101/cshperspect.a028241>.
- Cafferata, E.G.A., Guerrico, A.M.G., Pivetta, O.H., Santa-Coloma, T.A., 2001. NF-κB activation is involved in regulation of cystic fibrosis transmembrane conductance regulator (CFTR) by interleukin-1β. *J. Biol. Chem.* 276, 15441–15444. <https://doi.org/10.1074/jbc.M010061200>.
- Chanoux, R.A., Rubenstein, R.C., 2012. Molecular chaperones as targets to circumvent the CFTR defect in cystic fibrosis. *Front. Pharmacol.* 3. <https://doi.org/10.3389/fphar.2012.00137>.
- Collawn, J.F., Matalon, S., 2014. CFTR and lung homeostasis. *Am. J. Phys. Lung Cell. Mol. Phys.* 307, L917–L923. <https://doi.org/10.1152/ajplung.00326.2014>.
- Cordovado, S.K., Hendrix, M., Greene, C.N., Mochal, S., Earley, M.C., Farrell, P.M., Kharrazi, M., Hannon, W.H., Mueller, P.W., 2012. CFTR mutation analysis and haplotype associations in CF patients. *Mol. Genet. Metab.* 105, 249–254. <https://doi.org/10.1016/j.ymgme.2011.10.013>.
- de W. Groot, K.M., van der Ent, C.K., 2005. Nitric oxide in cystic fibrosis. *J. Cyst. Fibros.* 4, 25–29. <https://doi.org/10.1016/j.jcf.2005.05.008>.
- Dinh-Xuan, A.T., Hua-Huy, T., 2015. Should we monitor exhaled NO to assess the restoration of CFTR function in CF patients? *J. Cyst. Fibros.* 14, 683–684. [https://doi.org/10.1016/S1569-1993\(15\)00247-7](https://doi.org/10.1016/S1569-1993(15)00247-7).
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Hamacher, J., Hadizamani, Y., Borgmann, M., Mohaupt, M., Männel, D.N., Moehrlen, U., Lucas, R., Stammerger, U., 2018. Cytokine-ion channel interactions in pulmonary inflammation. *Front. Immunol.* 8. <https://doi.org/10.3389/fimmu.2017.01644>.
- Henke, M.O., John, G., Germann, M., Lindemann, H., Rubin, B.K., 2007. MUC5AC and MUC5B mucins increase in cystic fibrosis airway secretions during pulmonary exacerbation. *Am. J. Respir. Crit. Care Med.* 175, 816–821. <https://doi.org/10.1164/rccm.200607-1011OC>.
- Lazarowski, E.R., Boucher, R.C., 2009. Purinergic receptors in airway epithelia. *Curr. Opin. Pharmacol.* 9, 262–267. <https://doi.org/10.1016/j.coph.2009.02.004>.

- Lukowski, S.W., Rothnagel, J.A., Trezise, A.E.O., 2015. CFTR mRNA expression is regulated by an upstream open reading frame and RNA secondary structure in its 5' untranslated region. *Hum. Mol. Genet.* 24, 899–912. <https://doi.org/10.1093/hmg/ddu501>.
- McCarron, A., Donnelley, M., Parsons, D., 2018. Airway disease phenotypes in animal models of cystic fibrosis. *Respir. Res.* 19. <https://doi.org/10.1186/s12931-018-0750-y>.
- Mutlu, G.M., Factor, P., 2008. Alveolar epithelial beta2-adrenergic receptors. *Am. J. Respir. Cell Mol. Biol.* 38, 127–134. <https://doi.org/10.1165/rcmb.2007-0198TR>.
- Namkung, W., Finkbeiner, W.E., Verkman, A.S., 2010. CFTR-adenylyl cyclase I association responsible for UTP activation of CFTR in well-differentiated primary human bronchial cell cultures. *Mol. Biol. Cell* 21, 2639–2648. <https://doi.org/10.1091/mbc.E09-12-1004>.
- Poghosyan, A., Patel, J.K., Clifford, R.L., Knox, A.J., 2016. Epigenetic dysregulation of interleukin 8 (CXCL8) hypersecretion in cystic fibrosis airway epithelial cells. *Biochem. Biophys. Res. Commun.* 476, 431–437. <https://doi.org/10.1016/j.bbrc.2016.05.140>.
- Rowntree, R.K., Harris, A., 2003. The phenotypic consequences of CFTR mutations. *Ann. Hum. Genet.* 67, 471–485.
- Viart, V., Varilh, J., Lopez, E., René, C., Claustres, M., Taulan-Cadars, M., 2013. Phosphorylated C/EBP $\beta$  influences a complex network involving YY1 and USF2 in lung epithelial cells. *PLoS One* 8, e60211. <https://doi.org/10.1371/journal.pone.0060211>.
- Watson, M.J., Worthington, E.N., Clunes, L.A., Rasmussen, J.E., Jones, L., Tarhan, R., 2011. Defective adenosine-stimulated cAMP production in cystic fibrosis airway epithelia: a novel role for CFTR in cell signaling. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 25, 2996–3003. <https://doi.org/10.1096/fj.11-186080>.
- Weiler, C.A., Drumm, M.L., 2013. Genetic influences on cystic fibrosis lung disease severity. *Front. Pharmacol.* 4, 40. <https://doi.org/10.3389/fphar.2013.00040>.
- Xu, Y., Szép, S., Lu, Z., 2009. The antioxidant role of thiocyanate in the pathogenesis of cystic fibrosis and other inflammation-related diseases. *Proc. Natl. Acad. Sci. U. S. A.* 106, 20515–20519. <https://doi.org/10.1073/pnas.0911412106>.
- Zhang, Z., Leir, S.-H., Harris, A., 2015. Oxidative stress regulates CFTR gene expression in human airway epithelial cells through a distal antioxidant response element. *Am. J. Respir. Cell Mol. Biol.* 52, 387–396. <https://doi.org/10.1165/rcmb.2014-0263OC>.
- Ziady, A.G., Hansen, J., 2014. Redox balance in cystic fibrosis. *Int. J. Biochem. Cell Biol.* 113–123. <https://doi.org/10.1016/j.biocel.2014.03.006>.

# Diseases of the nervous system



## OUTLINE

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The nervous system is a complex network that transmits signals between the brain and spinal cord and various parts of the body. The central nervous system (CNS) consists of the brain and spinal cord, while the peripheral nervous system (PNS) consists mainly of nerves that connect the brain and spinal cord to every other organ. The neuron, a highly specialized cell with an electrically excitable membrane, is the primary functional entity of the nervous system.

Despite the efforts of researchers all over the world, the mechanisms underlying the pathology of neurological diseases remain unclear. In this chapter, we review the molecular pathogenesis of Alzheimer's disease and amyotrophic lateral sclerosis because these disorders are among the most challenging for both treatment and the development of effective drugs.

Alzheimer's disease is an example of a degenerative disease of the nervous system in which nerve tissues lose their function because of neuronal death or problems with structural and metabolic support, for example, dysfunction of glial cells.

Amyotrophic lateral sclerosis (ALS) is considered a systemic atrophy primarily affecting the CNS.

Episodic and paroxysmal disorders include different types of epilepsy and migraine. Familial hemiplegic migraine is reviewed in this chapter because it is an example of an inherited disease of the nervous system with known mutations in genes regulating neuronal and synaptic function. Studying inherited diseases of the nervous system on the molecular level make it possible to better understand nervous system pathology. However, it is difficult to study diseases of the nervous system on the molecular level due to a deficiency of tissue samples or of animal models. Linking molecular events in the nervous system with noticeable clinical manifestations is also a challenge for researchers.

There are other groups of diseases of nervous system that are not included in this chapter. They include inflammatory diseases of CNS and extrapyramidal and movement disorders such as Parkinson's disease and demyelinating diseases of the CNS such as multiple sclerosis. Different variants of stroke, including cerebral infarction due to thrombosis or embolism and intracerebral hemorrhage, which are the most common diseases that severely affect nervous system function, are classified as diseases of the circulatory system by the World Health Organization (<https://www.who.int/classifications/icd>).

## CHAPTER

## 5.1

## Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease associated with a steady progression of memory loss and higher cortical dysfunctions up to the total disintegration of intelligence and mental activity in general at the remote stages of the disease ([Burns and Iliffe, 2009](#)).

Dementia is a syndrome characterized by progressive loss of previously acquired cognitive skills including memory, language, insight, and judgment. Alzheimer's disease (AD) is believed to account for the majority (50%-75%) of all cases of dementia. ([Ferri and Ferri, 2018](#)).

Alzheimer's disease is one of the most frequent diseases of people at the age of 70 years and older ([Lyketsos, 2000](#)). In the initial stages of the disease, forgetfulness of recent events is the typical and often the only symptom. Among other early symptoms of Alzheimer's disease are the progressive decrease in the ability to generalize and understand written text, visual-spatial impairments, and attention deficit. Frequent symptoms of Alzheimer's disease include emotional-affective disorders and primarily depression, which is gradually decreased in advanced stages ([Jorm, 2001](#)). Once mild memory impairments are observed in patients with Alzheimer's disease, dementia develops within 6–7 years. As the disease progresses, the neurodegenerative process affects the frontal brain, and self-maintenance becomes difficult.

Studies of the pathogenesis of Alzheimer's disease indicate that Alzheimer's disease is heterogeneous regarding pathogenic mechanisms and causes ([Alzheimer's Association Report, 2016](#)). Still, the central and most widely discussed hypothesis is based on intracerebral amyloid deposition in the brain resulting in the progressive death of neurons. The formation of amyloid aggregates in neurons is associated with mutations in the genes encoding the proteolytic enzymes presenilins 1 and 2 (PSEN1 and PSEN2), which normally generate amyloid-beta peptides and mutations in the gene encoding the amyloid-beta precursor protein (APP). To date, more than 150 different mutations within these genes associated with familial cases of Alzheimer's disease have been described ([Wang et al., 2017](#)). Mutations in APP, PSEN1, and PSEN2 account for most cases

of early-onset familial Alzheimer's disease. However, the vast majority of Alzheimer's disease comprises the late-onset sporadic forms that cannot be explained by defects in these genes. The discovery of reliable polymorphisms associated with the late-onset form of Alzheimer's disease and risk biomarkers for the progression of mild cognitive impairment (MCI) into AD is the foci of current research. For example, an APOE4 allele is considered to be the primary genetic biomarker for late-onset development (Lacour et al., 2017; Pimenova et al., 2018; Scheltens et al., 2016).

Additional important triggers of Alzheimer's disease include accumulative oxidative stress, misfolded protein stress, Tau neurofibrillary tangles, and endosomal recycling of membrane receptors with lipoproteins particles (Di Domenico et al., 2017).

The molecular mechanisms of the amyloid synthesis and degradation have been studied intensively, but there are still many open questions about the pathophysiology of Alzheimer's disease. The role of mutated proteins that are involved in the synthesis and secretion of neurotoxic amyloid-beta peptides constitutes the basis of the signaling models of AD.

**Pathway 1. Amyloid-beta in Alzheimer's disease.**

The actual pathways involving amyloid-beta in neurons in AD are poorly understood. Mitochondrial damage and dysfunction due to amyloid-peptide toxicity has been examined.

APP processing and neurotoxic amyloid-beta formation (Fig. 1A);  
Intracellular amyloid-mediated neurotoxicity in AD (Fig. 1B).

Pernicious effects of amyloid plaque accumulation in the extracellular matrix of the brain also may account for the progressive neurodegenerative dementia in AD.

Extracellular amyloid-mediated effects in AD (Fig. 2A).

Alterations in the clearance and degradation of amyloid are considered the next important step in the complex process of the AD development. Modifications in apolipoprotein profiles and mutations in genes that encode proteins that are important for amyloid transport and clearance have strong associations with late-onset AD.

Amyloid-beta aggregates clearance (Fig. 2B).

## Key cellular contributors and processes

Complement system

Process

The complement system is a group of small proteins that “complement” the ability of the system to eliminate cellular pathogens. Proteins of the complement system, produced by the liver and circulating in the blood as inactive precursors, promote inflammation and attack the pathogen’s plasma membrane.

Oxidative stress

Process

Oxidative stress occurs when the antioxidant defense system is unable to neutralize the harmful effects of ROS.

Protein aggregation

Process

Protein aggregation is a biological process in which proteins with abnormal secondary or tertiary structures accumulate and stick together to form organized aggregates. The aggregation process is associated with a variety of health conditions including many neurodegenerative diseases in addition to Alzheimer’s disease.

Reactive oxygen species

Reactive oxygen species (ROS) are chemically reactive oxygen containing molecules that are commonly produced during normal metabolic processes involving oxygen. ROS can damage all essential cellular components including lipids, proteins, and DNA.

Synapse

The synapse is a specialized connection between two neurons or between a neuron and an effector cell where a nerve impulse can be conducted between the two cells.

## Pathway 1

### Amyloid-beta production and functions in Alzheimer's disease

#### Incoming signals

Amyloid-beta precursor protein (APP) is the central protein in Alzheimer's disease. The cleavage of APP forms two amyloid-beta (A-beta) peptides that are 40 and 42 amino acid residues long and that may oligomerize into complexes and aggregates causing neuritic plaque formation within neurons and in the extracellular space.

Mutations in APP and in the proteins that regulate APP endocytosis and processing in neurons enhance A-beta peptide synthesis and release and disturb APP-related intracellular signaling pathways. Dysfunction of the proteins that regulate amyloid plaque degradation, transport, and internalization can also cause Alzheimer's disease.

#### Outcome

A key aspect of the amyloidogenic hypothesis of Alzheimer's disease development is the neurodegenerative effect of soluble forms of A-beta and amyloid fibrils that are assembled inside and outside of neurons in the patients' brains. The mechanisms by which amyloid-beta may lead to neuronal loss are not yet clear but experimental evidence points to its toxicity to synapses and microglial function. The precise role of APP or A-beta peptides neurotoxicity regarding intracellular signaling pathways is not well established. Different hypotheses were explored including the pathways of damage to and enlargement of mitochondria ([Lopez Sanchez et al., 2017](#)).

Normally, APP participates in the regulation of different neuronal processes, such as neuroblast migration, synaptic growth, and remodeling after injury. APP integrates into the neuronal membrane and may function as both a protein of cell-to-cell adhesion and a membrane receptor. Probably, APP is a variant of a noncanonical G protein-coupled receptor, which can promote several signaling pathways and play a role in the complex cellular response to extracellular signals. In this case, amyloid-beta and other factors hyperactivate APP signaling to result in the neurodegenerative response and AD ([Ramaker and Copenhaver, 2017](#)).

#### Signaling

##### (a) APP processing and neurotoxic amyloid-beta formation (Fig. 1A)

Alternative splicing of the primary APP transcript produces three major isoforms containing the A-beta sequence. The two larger isoforms consist of 751 and 770 amino acid residues (APP751 and APP770, respectively). They contain a Kunitz protease inhibitor (KPI) domain and are

ubiquitously expressed. The third isoform, APP695, does not contain a KPI domain and is predominantly expressed in neurons.

The processing and cleavage of APP isoforms is normal and occurs mainly in cellular vesicles, specifically in the membranes of endosomes.

The first step of APP cleavage is performed by the alpha-secretases. This scenario of APP processing does not include the formation of amyloid peptides and thus is called a nonamyloidogenic process. APP is initially cleaved by alpha-secretase (ADAM9, ADAM10, and ADAM17) to generate a carboxy-terminal fragment 83 amino acids in length (C83). Then C83 can also undergo cleavage by gamma-secretase into the peptide P3 and the APP intracellular domain (AICD).

The rest of the APPs are initially cleaved by beta-secretase (BACE1) according to the alternative amyloidogenic scenario, releasing a C89 carboxyl-terminal fragment and retaining the last 99 amino acids of APP (C99) within the cell membrane. C99 is subsequently cleaved by gamma-secretase to produce amyloid-beta (A-beta) peptides of 40 and 42 amino acid residues, with the ratio of 10:1, respectively.

Also, theta-secretase (BACE2) and eta-secretase (MMP24) can cleave APP to release C80 and C191 fragments accordingly (Wang et al., 2017).

The gamma-site cleavage mechanism is imprecise so A-beta peptides of varying lengths can be formed. A-beta 40 is the most abundant peptide. In AD, mutated APP or mutated proteins of the gamma-secretase complex cause the release of higher levels of the 42 amino acid amyloidogenic peptide. The increased ratio of A-beta 42/A-beta 40 was suggested to be a critical point in the AD development (Zhang et al., 2011).

Many proteins are involved in APP processing, intracellular transport, and endocytic recycling. Malfunction of these proteins or disruption of those pathways that regulate endosome trafficking is critical steps for amyloid-beta formation and AD development (Wang et al., 2017). For example, sortilin-related receptor L (SORL1) interacts with APP in the Golgi complexes, and it protects APP from processing. In the absence of SORL1 or when its function is reduced, APP is delivered to a late endosome where it can be cleaved to produce A-beta peptides (Andersen et al., 2005; Pimenova et al., 2018). On the other hand, there are receptors, for example, low-density lipoprotein receptor class A domain containing 3 (LRAD3) and lipoprotein receptor 1 (LPR1), that normally promote APP endocytosis and processing (Lane-Donovan et al., 2014).

### (b) Intracellular amyloid-beta-mediated neurotoxicity (Fig. 1B)

Amyloid-beta peptides can exist in multiple states of assembly—monomers, oligomers, protofibrils, and fibrils. Monomeric A-beta is soluble, but oligomeric and cross-linked A-beta are not, ultimately generating highly insoluble and stable plaques. The process of A-beta assembly has been studied in detail (Bohm et al., 2015; Knowles et al., 2014).

A-beta 42 is more hydrophobic and more prone to assembly into oligomers or fibrils. Diffuse plaques are not fibrillar and consist almost completely of A-beta 42. Compact neuritic amyloid plaques that are specific for AD contain both A-beta 40/42 fibrillar deposits ([Irvine et al., 2008](#)).

Soluble oligomers inside neurons are highly neurotoxic, although the exact mechanism of their toxicity is still not clear. Several hypotheses were suggested; the formation of microtubule-associated protein Tau-related neurofibrillary tangles (NFTs), and mitochondrial decline are ones of the most discussed.

In Alzheimer's disease, dysfunction of mitochondria occurs in neurons. Aspects of mitochondrial dysfunction are as follows: Mitochondria release higher amounts of reactive oxygen species (ROS) into the cytoplasm, the mitochondrial respiratory chain produces lower amounts of ATP, and mitochondria extend in size. These abnormalities trigger oxidative stress and apoptosis, resulting in the degeneration and death of neurons. The generation of reactive oxygen species (ROS) is probably one of the key features of neuronal death in Alzheimer's disease. Mitochondrial decline, the reaction between amyloid-beta and redox-active metals, and calcium excitotoxicity may cause the generation of ROS in AD. Peroxidation of lipids in cellular membranes is one of the toxic effects of ROS. In turn, lipid peroxidation causes the release of toxic 4-hydroxynonenal or propanedial that may in turn cause cellular necrosis ([Godoy et al., 2014](#)). Also, a calcium overload is observed in Alzheimer's disease. Calcium overload probably results from the opening of the voltage-gated calcium channels (VGCCs) when A-beta binds to them. Calcium overload promotes both oxidative stress and mitochondrial damage ([Kawahara et al., 2009](#); [Small et al., 2009](#)).

Calcium overload and a decline in mitochondrial function result in low levels of glucose and reduced energy metabolism in neurons that leads to neuronal death and a progressive decrease of synaptic activity. The enlargement of mitochondria associated with Alzheimer's disease and their dysfunction are similar to that observed in aging neurons. It is not clear whether mitochondrial dysfunction causes Alzheimer's disease or represents one of its consequences ([Godoy et al., 2014](#)).

Not only amyloid-beta but also the APP intracellular domain (AICD), a product of both nonamyloidogenic and amyloidogenic processing, influence neuronal state. AICD interacts with several proteins such as lysine acetyltransferase 5 (KAT5), and it regulates transcription in the cell's nucleus. Probably, AICD is involved in the interaction with multifunctional G protein signaling. If APP processing becomes chronically hyperactivated, intensive activation of intracellular signaling pathways may result in neuronal depletion ([Ramaker and Copenhagen, 2017](#)).

APP and its derivatives are probably also involved in the regulation of expression of known cerebrospinal fluid biomarkers of the neurodegeneration in AD, such as neurogranin (NRGN) or T-tau (MART) ([Blennow, 2017](#); [Mattsson et al., 2016](#)).

The accumulation of an abnormal form of MAPT inside neurons is believed to contribute to the neuronal damage characteristic of Alzheimer's disease. Triggered by calcium overload and apoptosis, MAPT can aggregate in neurofibrillary tangles (NFTs), which in turn impair cytoskeleton organization and disrupt neuronal intracellular trafficking. An association between APP, amyloid, and NFT formation has been proposed. Amyloid-beta peptides or AICD may cause calcium overload or result in high level of GSK3B expression in neurons, which in turn phosphorylates MART. The hyperphosphorylated MAPT dissociates from microtubules, self-aggregates, binds MAP2, and accumulates in the cytoplasm to form NFTs (Bakota and Brandt, 2016; Panza et al., 2016).

(c) Extracellular amyloid-beta-mediated toxicity (Fig. 2A)

The main aspect of the amyloidogenic hypothesis of Alzheimer's disease involves the harmful effects of the physical aggregation of amyloid fibrils that are assembled in the extracellular space of patients' brains. The amyloid plaques physically alter synaptic function. The accumulation of amyloid-beta is believed to interfere with neuron-to-neuron communication at synapses so that information transfer at synapses begins to fail and the number of synapses declines.

Amyloid monomers outside of neurons can also be toxic. In the early stages of Alzheimer's disease, monomeric A-beta may protect neurons by binding to potentially toxic metal anions such as Cu(II) that were released at the synapse. The long-term oligomeric A-beta could concentrate the redox-active form of Cu(II) to form nonfibrillary amorphous aggregations of A-beta. Zn(II), Fe(II), and Fe(III) also bind to A-beta to accelerate its aggregation. Anions accumulated in plaques can participate in Fenton reactions to form reactive oxygen species, which cause lipid peroxidation or to perturb calcium homeostasis and cause synaptic dysfunction (Jiang et al., 2009; Maynard et al., 2005; Russo et al., 2002).

Moreover, it is known that the presence of extracellular amyloid oligomers and fibrils activates the innate immune response for removing misfolded proteins. The chronic activation of complement receptor (CR1) and microglial TREM2 is important for this protective response. However, details of the relationship between amyloid and different cell types remain unclear. Probably, after some critical time point, the removal of amyloid is no longer sufficient to stop the progression of the neurodegeneration (Bohm et al., 2015; Knowles et al., 2014).

(d) Amyloid-beta aggregates clearance (Fig. 2B)

Degradation of fibrillar A-beta can be accomplished via two major pathways: proteolytic degradation and receptor-mediated transport out of the brain.

A family of low-density lipoprotein receptors and chaperone molecules are involved in a receptor-mediated efflux of A-beta peptides across the

blood-brain barrier and the internalization of amyloid by neurons, astrocytes, microglia, and cells of the innate immune system.

The chaperone molecules apolipoprotein E (APOE), alpha-2-macroglobulin (A2M), or clusterin (CLU) are required for soluble amyloid trafficking into neurons and astrocytes.

Mutations in the gene encoding the apolipoprotein E (APOE) isoform 4 (APOE4) are the major known genetic risk factors for late-onset Alzheimer's disease. APOE in the brain is synthesized and secreted primarily by astrocytes. APOE isoforms (APOE2, APOE3, and APOE4) transport lipids throughout the brain, providing material and energy for neuron myelination and remodeling.

A person who only inherits a specific isoform of APOE4 has a greater chance to develop AD, probably, because this isoform has an unstable structure compared with APOE2 and APOE3 and its activity can be insufficient for effective lipid trafficking. There is also evidence that APOE4 is the worst carrier among all APOE isoforms with regard to A-beta 42 clearance in APOE-lipid particles.

Apolipoprotein J (clusterin, CLU) is another chaperone involved in the clearance of amyloid-beta peptides. The levels of CLU were elevated in AD, and mutations in the CLU gene have strong associations with rapid AD progression. CLU has many other functions and might play the central role in AD development along with APOE (Misra et al., 2018; Nuutinen et al., 2009).

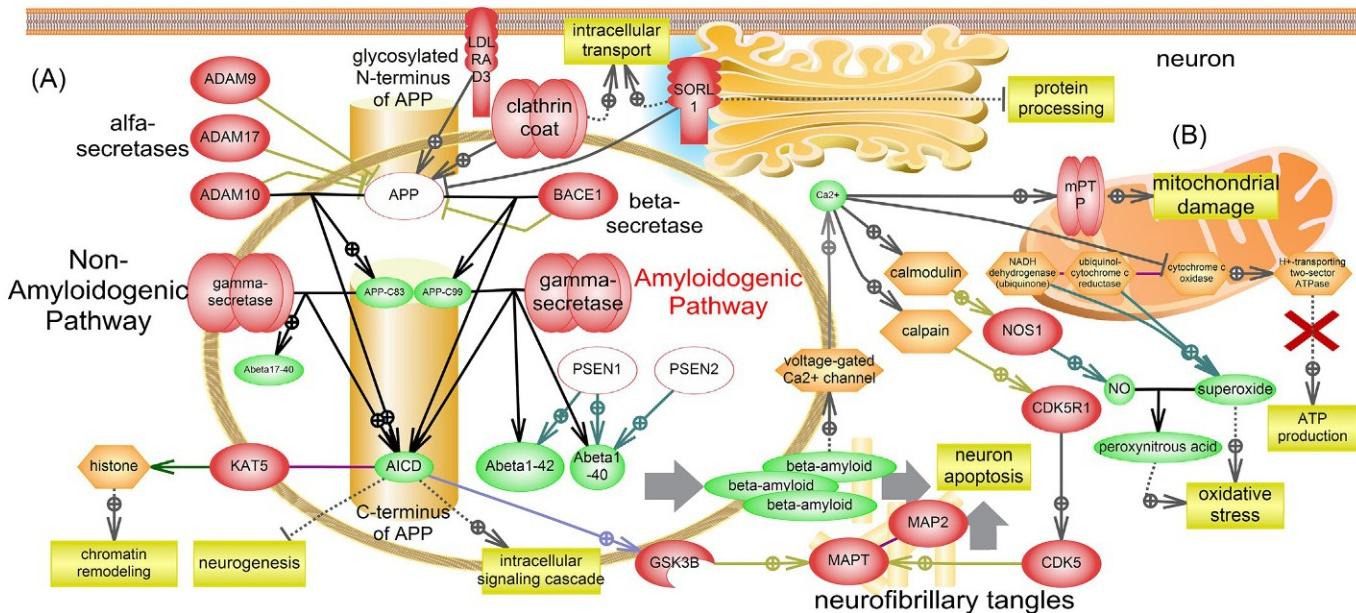
APOE particles with amyloid can be internalized by neurons, astrocytes, microglia, and other cells, which express LDLRs (Bales, 2010). Clathrin-mediated endocytosis is the typical mechanism of amyloid-beta internalization. APOE-lipid particles bind to beta-amyloid and get absorbed by endocytosis mediated by the low-density lipoprotein receptor (LDLR) family.

The low-density lipoprotein receptor-related protein 1 (LRP1) and the receptors for Reelin (RELN) are required for amyloid-beta endocytosis in several cell types. RELN is involved with neuron receptor endosomal recycling and, in general, has a neuroprotective role. APOE4 also binds to RELN receptors (LRP8, VLDLR), thereby blocking Reelin's ability to protect the synapse against amyloid uptake (Bock and May, 2016; Lane-Donovan et al., 2014).

In astrocytes, the formyl peptide receptor 2 (FPR2 or FPRL1), the nicotinic acetylcholine receptors, and others mediate A-beta internalization.

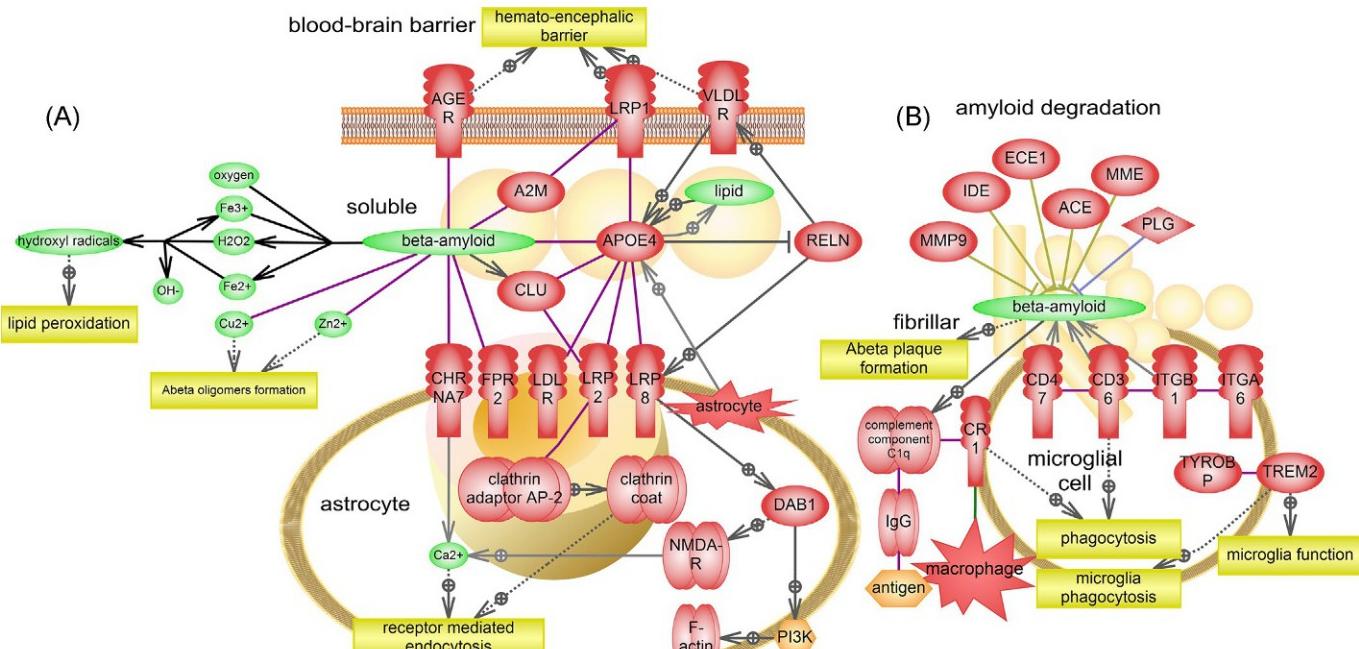
Microglia degrade and uptake soluble amyloid independent of APOE before receptor-associated endocytosis.

The flux of A-beta across the blood-brain barrier is maintained by receptor for advanced glycation end products (RAGE) and chaperone molecules. Once A-beta enters the bloodstream, it can either reenter the brain via the RAGE receptor or be delivered to peripheral sites of degradation (e.g., to the liver or kidney) by chaperone molecules (Mohamed and Posse de Chaves, 2011).



**FIG. 1** Pathway 1: Amyloid-beta in Alzheimer's disease. APP processing and neurotoxic amyloid-beta formation (A). Intracellular amyloid-mediated neurotoxicity in AD (B).

## II. Human disease pathways



**FIG. 2** Pathway 1: Amyloid-beta in Alzheimer's disease. Extracellular amyloid-mediated effects in AD (A). Amyloid-beta aggregates clearance (B).

## References

- Disease number #104300 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code G30. Diseases of the nervous system (G00-G99). (ICD-10, <https://icdlist.com>).
- Alzheimer's Association Report, 2016. 2016 Alzheimer's disease facts and figures. *Alzheimers Dement.* 12, 459–509. <https://doi.org/10.1016/j.jalz.2016.03.001>.
- Andersen, O.M., Reiche, J., Schmidt, V., Gotthardt, M., Spoelgen, R., Behlke, J., von Arnim, C.A.F., Breiderhoff, T., Jansen, P., Wu, X., Bales, K.R., Cappai, R., Masters, C.L., Gliemann, J., Mufson, E.J., Hyman, B.T., Paul, S.M., Nykjaer, A., Willnow, T.E., 2005. Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein. *Proc. Natl. Acad. Sci.* 102, 13461–13466. <https://doi.org/10.1073/pnas.0503689102>.
- Bakota, L., Brandt, R., 2016. Tau biology and tau-directed therapies for Alzheimer's disease. *Drugs* 76, 301–313. <https://doi.org/10.1007/s40265-015-0529-0>.
- Bales, K.R., 2010. Brain lipid metabolism, apolipoprotein E and the pathophysiology of Alzheimer's disease. *Neuropharmacology* 59, 295–302. <https://doi.org/10.1016/j.neuropharm.2010.01.005>.
- Blennow, K., 2017. A review of fluid biomarkers for Alzheimer's disease: moving from CSF to blood. *Neurol. Ther.* 6, 15–24. <https://doi.org/10.1007/s40120-017-0073-9>.
- Bock, H.H., May, P., 2016. Canonical and non-canonical Reelin signaling. *Front. Cell. Neurosci.* 10, 166. <https://doi.org/10.3389/fncel.2016.00166>.
- Bohm, C., Chen, F., Sevalle, J., Qamar, S., Dodd, R., Li, Y., Schmitt-Ulms, G., Fraser, P.E., St George-Hyslop, P.H., 2015. Current and future implications of basic and translational research on amyloid- $\beta$  peptide production and removal pathways. *Mol. Cell. Neurosci.* 66, 3–11. <https://doi.org/10.1016/j.mcn.2015.02.016>.
- Burns, A., Iliffe, S., 2009. Alzheimer's disease. *BMJ* 338, b158. <https://doi.org/10.1136/bmj.b158>.
- Di Domenico, F., Barone, E., Perluigi, M., Butterfield, D.A., 2017. The triangle of death in Alzheimer's disease brain: the aberrant cross-talk among energy metabolism, mammalian target of rapamycin signaling, and protein homeostasis revealed by redox proteomics. *Antioxid. Redox Signal.* 26, 364–387. <https://doi.org/10.1089/ars.2016.6759>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Godoy, J.A., Rios, J.A., Zolezzi, J.M., Braidy, N., Inestrosa, N.C., 2014. Signaling pathway cross talk in Alzheimer's disease. *Cell Commun. Signal.* 12, 23. <https://doi.org/10.1186/1478-811X-12-23>.
- Irvine, G.B., El-Agnaf, O.M., Shankar, G.M., Walsh, D.M., 2008. Protein aggregation in the brain: the molecular basis for Alzheimer's and Parkinson's diseases. *Mol. Med.* 14, 451–464. <https://doi.org/10.2119/2007-00100.Irvine>.
- Jiang, D., Li, X., Williams, R., Patel, S., Men, L., Wang, Y., Zhou, F., 2009. Ternary complexes of iron, amyloid-beta, and nitrilotriacetic acid: binding affinities, redox properties, and relevance to iron-induced oxidative stress in Alzheimer's disease. *Biochemistry* 48, 7939–7947. <https://doi.org/10.1021/bi900907a>.
- Jorm, A.F., 2001. History of depression as a risk factor for dementia: an updated review. *Aust. N. Z. J. Psychiatry* 35, 776–781. <https://doi.org/10.1046/j.1440-1614.2001.00967.x>.
- Kawahara, M., Negishi-Kato, M., Sadakane, Y., 2009. Calcium dyshomeostasis and neurotoxicity of Alzheimer's beta-amyloid protein. *Expert. Rev. Neurother.* 9, 681–693. <https://doi.org/10.1586/ern.09.28>.
- Knowles, T.P.J., Vendruscolo, M., Dobson, C.M., 2014. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* 15, 384–396. <https://doi.org/10.1038/nrm3810>.

- Lacour, A., Espinosa, A., Louwersheimer, E., Heilmann, S., Hernández, I., Wolfsgruber, S., Fernández, V., Wagner, H., Rosende-Roca, M., Mauleón, A., Moreno-Grau, S., Vargas, L., Pijnenburg, Y.a.L., Koene, T., Rodríguez-Gómez, O., Ortega, G., Ruiz, S., Holstege, H., Sotolongo-Grau, O., Kornhuber, J., Peters, O., Frölich, L., Hüll, M., Rüther, E., Wiltfang, J., Scherer, M., Riedel-Heller, S., Alegret, M., Nöthen, M.M., Scheltens, P., Wagner, M., Tárraga, L., Jessen, F., Boada, M., Maier, W., van der Flier, W.M., Becker, T., Ramirez, A., Ruiz, A., 2017. Genome-wide significant risk factors for Alzheimer's disease: role in progression to dementia due to Alzheimer's disease among subjects with mild cognitive impairment. *Mol. Psychiatry* 22, 153–160. <https://doi.org/10.1038/mp.2016.18>.
- Lane-Donovan, C.E., Philips, G.T., Herz, J., 2014. More than cholesterol transporters: lipoprotein receptors in CNS function and neurodegeneration. *Neuron* 83, 771–787. <https://doi.org/10.1016/j.neuron.2014.08.005>.
- Lopez Sanchez, M.I.G., Waugh, H.S., Tsatsanis, A., Wong, B.X., Crowston, J.G., Duce, J.A., Trounce, I.A., 2017. Amyloid precursor protein drives down-regulation of mitochondrial oxidative phosphorylation independent of amyloid beta. *Sci. Rep.* 7, 9835. <https://doi.org/10.1038/s41598-017-10233-0>.
- Lyketos, C.G., 2000. Mental and behavioral disturbances in dementia: findings from the Cache County study on memory in aging. *Am. J. Psychiatry* 157, 708–714. <https://doi.org/10.1176/appi.ajp.157.5.708>.
- Mattsson, N., Insel, P.S., Palmqvist, S., Portelius, E., Zetterberg, H., Weiner, M., Blennow, K., Hansson, O., 2016. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol. Med.* 8, 1184–1196. <https://doi.org/10.15252/emmm.201606540>.
- Maynard, C.J., Bush, A.I., Masters, C.L., Cappai, R., Li, Q.-X., 2005. Metals and amyloid-beta in Alzheimer's disease. *Int. J. Exp. Pathol.* 86, 147–159. <https://doi.org/10.1111/j.0959-9673.2005.00434.x>.
- Misra, A., Chakrabarti, S.S., Gambhir, I.S., 2018. New genetic players in late-onset Alzheimer's disease: findings of genome-wide association studies. *Indian J. Med. Res.* 148, 135–144. [https://doi.org/10.4103/ijmr.IJMR\\_473\\_17](https://doi.org/10.4103/ijmr.IJMR_473_17).
- Mohamed, A., Posse de Chaves, E., 2011. A $\beta$  internalization by neurons and glia. *Int. J. Alzheimers Dis.* 2011, 127984. <https://doi.org/10.4061/2011/127984>.
- Nuutinen, T., Suuronen, T., Kauppinen, A., Salminen, A., 2009. Clusterin: a forgotten player in Alzheimer's disease. *Brain Res. Rev.* 61, 89–104. <https://doi.org/10.1016/j.brainresrev.2009.05.007>.
- Panza, F., Solfrizzi, V., Seripa, D., Imbimbo, B.P., Lozupone, M., Santamato, A., Zecca, C., Barulli, M.R., Bellomo, A., Pilotto, A., Daniele, A., Greco, A., Logroscino, G., 2016. Tau-centric targets and drugs in clinical development for the treatment of Alzheimer's disease. *Biomed. Res. Int.* 2016. <https://doi.org/10.1155/2016/3245935>.
- Pimenova, A.A., Raj, T., Goate, A.M., 2018. Untangling genetic risk for Alzheimer's disease. *Biol. Psychiatry* 83, 300–310. <https://doi.org/10.1016/j.biopsych.2017.05.014>.
- Ramaker, J.M., Copenhagen, P.F., 2017. Amyloid Precursor Protein family as unconventional Go-coupled receptors and the control of neuronal motility. *Neurogenesis* 4, e1288510. <https://doi.org/10.1080/23262133.2017.1288510>.
- Russo, C., Dolcini, V., Salis, S., Venezia, V., Violani, E., Carlo, P., Zambrano, N., Russo, T., Schettini, G., 2002. Signal transduction through tyrosine-phosphorylated carboxy-terminal fragments of APP via an enhanced interaction with Shc/Grb2 adaptor proteins in reactive astrocytes of Alzheimer's disease brain. *Ann. N. Y. Acad. Sci.* 973, 323–333.
- Scheltens, P., Blennow, K., Breteler, M.M.B., de Strooper, B., Frisoni, G.B., Salloway, S., Van der Flier, W.M., 2016. Alzheimer's disease. *Lancet* 388, 505–517. [https://doi.org/10.1016/S0140-6736\(15\)01124-1](https://doi.org/10.1016/S0140-6736(15)01124-1).
- Small, D.H., Gasperini, R., Vincent, A.J., Hung, A.C., Foa, L., 2009. The role of Abeta-induced calcium dysregulation in the pathogenesis of Alzheimer's disease. *J. Alzheimers Dis.* 16, 225–233. <https://doi.org/10.3233/JAD-2009-0951>.

- Wang, X., Zhou, X., Li, G., Zhang, Y., Wu, Y., Song, W., 2017. Modifications and trafficking of APP in the pathogenesis of Alzheimer's disease. *Front. Mol. Neurosci.* 10. <https://doi.org/10.3389/fnmol.2017.00294>.
- Zhang, Y., Thompson, R., Zhang, H., Xu, H., 2011. APP processing in Alzheimer's disease. *Mol. Brain* 4, 3. <https://doi.org/10.1186/1756-6606-4-3>.

## CHAPTER

## 5.2

## Amyotrophic lateral sclerosis

In amyotrophic lateral sclerosis (ALS) motor neurons in the brain and spinal cord die (atrophy) over time, leading to muscle weakness, a loss of muscle mass, and an inability to control movement (ICD-10, <https://icdlist.com>). ALS types are distinguished by their clinical manifestations, genetic cause, or lack of clear genetic association. In many cases, ALS is a life-threatening disease. Death from respiratory failure often follows 3 years after disease onset.

Amyotrophic lateral sclerosis (ALS) is a progressive, degenerative neuromuscular condition of undetermined etiology affecting corticospinal tracts and anterior horn cells, resulting in dysfunction of both upper motor neurons (UMN) and lower motor neurons (LMN), respectively. (*Ferri and Ferri, 2018*).

Most incidents (approximately 90%) of ALS are sporadic with no apparent family history of the disease, and around 10% are familial. Sporadic ALS usually develops between the ages of 40 and 70 years (Genetics Home Reference, <https://ghr.nlm.nih.gov>). Sporadic ALS is a multifactorial disease that involves different cellular mechanisms of neuron toxicity of unclear causes. Even familial ALS is a genetically heterogeneous disorder because many different mutations have been associated with hereditary ALS. For example, different mutations in the superoxide dismutase 1 (*SOD1*) gene have been identified among one quarter of patients with familial ALS. Although mutations in several genes have been discovered, the molecular mechanisms underlying the characteristic neuron toxicity are still poorly understood. There is evidence that skeletal muscle lipid hypermetabolism can trigger the progression of ALS in patients with an existing genetic susceptibility (*Desseille et al., 2017; Morello et al., 2018*).

The precise mechanism of ALS pathogenesis is still unknown. One hypothesis is that motor neurons can be damaged by glutamate overload.

**Pathway 1. Glutamate and calcium excitotoxicity of motor neurons in ALS (Fig. 3).**

Mutations in the proteins, which regulate mitochondrial function, endocytosis and the degradation of misfolded proteins, or RNA metabolism, can lead to the accumulation of toxic protein aggregates in neurons, which initiate neuron apoptosis.

**Pathway 2.** *Mutations in SOD1 and associated proteins cause motor neuron death in familial ALS (Fig. 4).*

## Key cellular contributors and processes

Endocytosis

Process

Endocytosis is a highly conserved biological process in eukaryotes by which a cell internalizes extracellular substances by engulfing them with its membrane in an energy-dependent manner. The major variations of endocytosis include phagocytosis (ingestion of larger particles) and pinocytosis (ingestion of fluids, macromolecules, or smaller particles).

Endoplasmic reticulum-associated protein degradation

Process

ERAD stands for endoplasmic reticulum-associated protein degradation, which is a cellular process that targets misfolded proteins for degradation by the cytoplasmic ubiquitin-proteasome system.

Excitotoxicity

Process

Excitotoxicity refers to neuronal cell damage or death due to excessive stimulation by neurotransmitters, such as glutamate and similar substances.

Motor neuron

Cell

A motor neuron is an efferent neuron (transmitting the impulse away from the brain or spinal cord) located in (1) the spinal cord whose axon projects outside the spinal cord (lower motor neuron) or (2) the motor cortex of the brain whose axon descends to the spinal cord (upper motor neurons). Motor neuron axons conduct signals to their effectors (mainly muscles or lower motor neurons) to produce effects.

Protein aggregation

Process

Protein aggregation is a biological process in which proteins with abnormal secondary or tertiary structures accumulate and stick together forming organized aggregates. The aggregation process is associated with a variety of health conditions including many neurodegenerative diseases.

Synapse

Anatomic structure

Synapse is a specialized connection between two neurons or between a neuron and an effector cell where a nerve impulse can be conducted between the two cells.

## Pathway 1

### Glutamate and calcium excitotoxicity of motor neurons in ALS (Fig. 3)

#### Incoming signals

A motor neuron cell body is located in the spinal cord, and its fiber (axon) projects outside the spinal cord to directly or indirectly control effector organs, mainly muscles and glands. There are upper motor neurons and lower motor neurons, with the cell type described earlier being a lower motor neuron. The axons of lower motor neurons are efferent nerve fibers that carry signals from the spinal cord to the effectors. Upper motor neurons are corticospinal interneurons that arise from the motor cortex of the brain and descend into the spinal cord where they activate lower motor neurons through synapses. The term “motor neuron” is usually restricted to the efferent nerves that innervate muscles, namely, the lower motor neurons.

Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS). Under normal physiological conditions, glutamate is released from presynaptic motor neurons via  $\text{Ca}^{2+}$ -dependent exocytosis to activate receptors expressed on postsynaptic motor neurons.

Lower (postsynaptic) motor neurons can die because of excessive stimulation caused by glutamate binding to their glutamate receptors. The molecular mechanisms and triggers of this type of neuronal injury (glutamate excitotoxicity) in ALS are not yet well established. Loss of function of the glial glutamate reuptake transporter (SLC1A2, GLT1), or the DAO enzyme can trigger glutamate excitotoxicity. In ALS models, SLC1A2 expression is low and results in the inhibition of effective glutamate reuptake by astrocytes ([Rosenblum et al., 2017](#)).

#### Outcome effects

In lower motor neurons, glutamate excitotoxicity raises intracellular  $\text{Ca}^{2+}$  levels, which in turn facilitates nitric oxide (NO) formation. Subsequently, peroxynitrite synthesis triggers damage to the lower motor neurons. The excessive influx of  $\text{Ca}^{2+}$  into mitochondria results in mitochondrial dysfunction and subsequent motor neuron apoptosis ([Ferraiuolo et al., 2011](#); [Foran and Trott, 2009](#); [Pasinelli and Brown, 2006](#); [Redler and Dokholyan, 2012](#)).

#### Signalling

The action of glutamate in the synapse is terminated by its rapid reuptake via glutamate transporter proteins, solute carrier family 1 members

(SLC1A3 and SLC1A2), which are expressed on glial cells and astrocytes. SLC1A1 is located mainly on the presynaptic motor neurons.

SLC1A2 proteins on glial cells and astrocytes are responsible for the re-uptake of most of the glutamate. In neurons of patients with sporadic ALS, there is a profound reduction in the expression and activity of SLC1A2 both in the cortex and the spinal cord, probably due to specific cleavage by caspase-3 (CASP3) (Rosenblum et al., 2017), although the precise causes of SLC1A2 dysfunction are not known. Perhaps the altered function of the HNRNPK protein (heterogeneous nuclear ribonucleoprotein K) may regulate SLC1A2 expression in ALS (Yang et al., 2009).

Increased levels of reactive oxygen species (ROS) may inhibit the function of SLC1A2 and SLC1A3. A high level of the 4-hydroxynonenal lipid peroxidation product has been found in the spinal cords of ALS patients. 4-Hydroxynonenal promotes the intermolecular cross-linking of SLC1A2 monomers to form nonfunctional dimers.

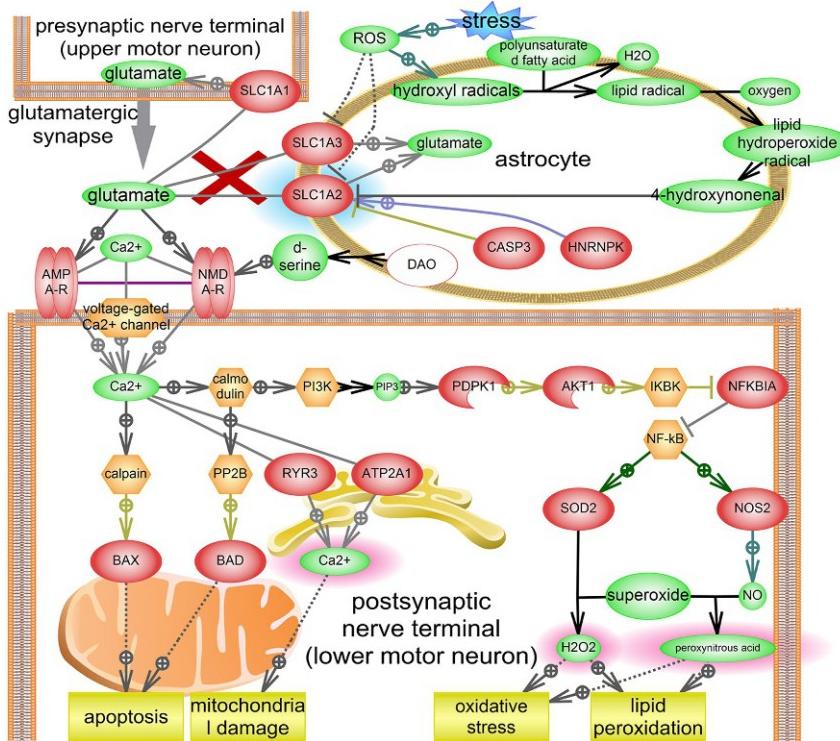
The inhibitory mutation R199W in D-amino acid oxidase (DAO) is associated with familial ALS. Glial cells expressing mutant DAO proteins induce apoptosis in lower motor neurons. Probably, in this case, mutant DAO may increase D-serine levels, which like glutamate, is an activator and coagonist of the N-methyl-D-aspartate (NMDA) receptor (Paul et al., 2014).

In lower motor neurons, glutamate activates quisqualic acid receptors (AMPA-R), the N-methyl-D-aspartate (NMDA) selective glutamate receptor, and voltage-gated  $\text{Ca}^{2+}$  channels, which together pump  $\text{Ca}^{2+}$  into the cell. The glutamate ionotropic receptor AMPA type subunit 1 (GluR2 or GRIA2) in other cells restricts  $\text{Ca}^{2+}$  permeability. Since this subunit is deficient in motor neurons, the AMPA receptors on motor neurons are unusually  $\text{Ca}^{2+}$  permeable and therefore vulnerable to excessive glutamate stimulation. Excessive  $\text{Ca}^{2+}$  release from the endoplasmic reticulum by the ryanodine receptor 3 (RYR3) accelerates intracellular  $\text{Ca}^{2+}$  overload.

In ALS neurons, mitochondria may be a critical intracellular target of injury after intense  $\text{Ca}^{2+}$  channel stimulation.

NO synthesis in postsynaptic neurons depends on calcium signaling and on NF- $\kappa$ B activation, which activates the transcription of NO synthases 2 (NOS2) and the potent oxidative enzyme, superoxide dismutase 2 (SOD2).

Further, peroxynitrite, a potent oxidant, is formed as a result of the reaction of superoxide radicals and NO.



**FIG. 3** Pathway 1: Glutamate and calcium excitotoxicity of motor neurons in ALS.

## Pathway 2

### Mutations in SOD1 and associated proteins cause motor neuron death in familial ALS (Fig. 4)

#### Incoming signals

About 20%–25% of familial ALS arise from the loss-of-function mutations in the superoxide dismutase 1 (*SOD1*) gene (Deselle et al., 2017; Morello et al., 2018). The SOD1 enzyme plays a vital role as a cellular antioxidant. The intentional loss of SOD1 function in animal models can selectively kill motor neurons.

A substitution of alanine for the glycine at position 93 in SOD1 (G93A) is the most studied single-nucleotide variant (SNV) in ALS. SOD1 (G93A) results in protein misfolding and leads to the formation of toxic insoluble aggregates, which impair multiple cellular functions.

Also, other rare ALS-associated mutations that dysregulate neuronal endosomal trafficking were described (Swarup and Julien, 2011). Rare mutations in the amyotrophic lateral sclerosis 2, juvenile (*ALS2*) gene are associated with a form of autosomal recessive juvenile-onset ALS (Sheerin et al., 2014; Webster et al., 2017).

Mutations in several other genes, which are responsible for RNA transcription, processing, splicing, DNA repair, or axonal cargo transport, are involved in the pathogenesis of ALS. An example includes mutations in the TAR DNA-binding protein (TARDBP) gene and the gene encoding the fused in sarcoma protein (FUS), which may form aggregates in SOD1-independent cases of ALS (Dewey et al., 2012; Farg et al., 2012).

#### Outcome effects

The accumulation of misfolded proteins in neurons triggers mitochondrial dysfunction, which includes the overloading of endoplasmic reticulum–associated degradation of proteins (ERAD), the unfolded protein response (UPR), and the impairment of autophagy. The mitochondrial dysfunction prominent in ALS neurons initiates apoptosis. All this leads to the further accumulation of insoluble protein aggregates, a reduction of transcription, and diminished DNA repair leading to neuron death (Boillée et al., 2006; Cozzolino et al., 2013; Ferraiuolo et al., 2011; Pasinelli and Brown, 2006; Redler and Dokholyan, 2012; Shi et al., 2010).

#### Signaling

Normally, SOD1 converts the superoxide anion to hydrogen peroxide ( $H_2O_2$ ) and protects the cell from oxidative stress. Mutant misfolded SOD1

(mSOD1) aggregates in the mitochondria and may directly damage them. Mutant SOD1 (mSOD1) directly binds to the voltage-dependent anion channel 1 (VDAC1) protein, depolarizes the mitochondrial membrane, and disrupts the normal functioning of the electron transport chain. Also, mSOD1 sequesters the antiapoptotic mitochondrial protein B-cell lymphoma protein 2 alpha (BCL2), which then aggregates within the mitochondria making them nonfunctional, thereby triggering the apoptosis. mSOD1 also impairs the association of cytochrome *c*, somatic (CYCS) with the inner membrane of the mitochondrion leading to the release of CYCS into the cytoplasm and the subsequent activation of the caspases. Also, mSOD1 participates in the activation of cytochrome *b*-245 (CYBB) or of the transcription factor TP53 along with caspase 1 (CASP1) activation, promoting both apoptosis and inflammation.

Misfolded SOD1 can be secreted from neurons with the help of chromogranin-A and chromogranin-B (CHGA/B), which are both abundant proteins in motor neurons and in interneurons.

Additionally, mSOD1 may impair the function of the mitochondrial protein translocation machinery (including the TOM and TIMM23 complexes) and limit the import of functional proteins into the mitochondria.

mSOD1 also impairs anterograde and retrograde axonal transport in motor neurons and endosomal trafficking in general. SOD1 interacts with kinesin-associated protein 3 (KIFAP3) and the dynein ATPase. DCTN1 functions as an adaptor between the dynein ATPase protein and various cargos, thereby regulating the efficiency of the dynein motor and taking part in axonal cargo transport. The DCTN1 gene may be mutated in some ALS cases ([Vilarino-Güell et al., 2009](#)).

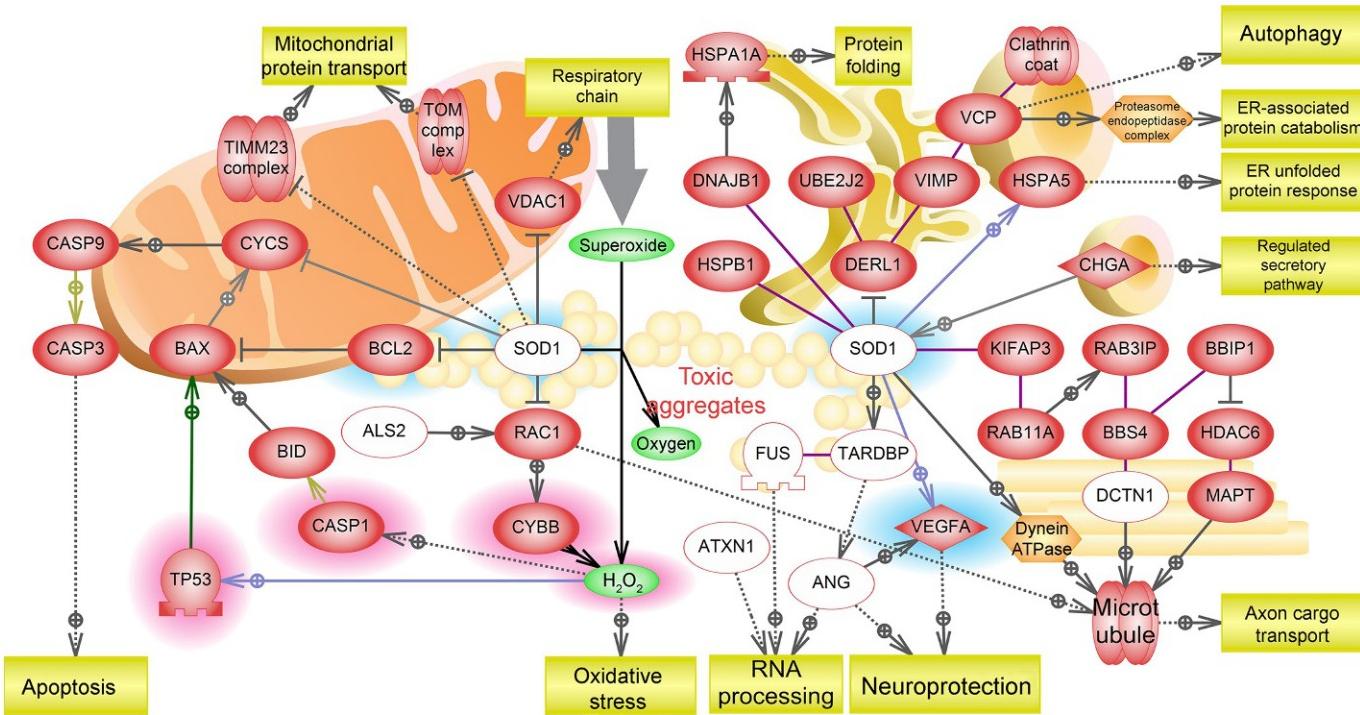
Overall protein quality, which is normally controlled by the endoplasmic reticulum-associated degradation (ERAD) mechanism, is impaired in ALS neurons. mSOD1 binds to derlin 1 (DERL1), a transmembrane protein responsible for the translocation of misfolded proteins from the ER lumen. Rare mutations in the ALS2 gene are associated with a form of autosomal recessive juvenile-onset ALS. The ALS2 protein interacts with RAB5A and RAC1 and is involved with endosomal fusion/trafficking and microtubule assembly. mSOD1 also interacts with members of the heat shock family of proteins such as HSPA5, HSPA1A, DNAJB1, and HSPB1 to impair the chaperone function of these proteins during protein processing within the Golgi and the ER.

Furthermore, mSOD1 may bind to the TAR DNA-binding protein 43 (TARDBP) causing it to aggregate, thereby disrupting normal RNA processing. Mutations in both TARDBP and FUS were found in patients with ALS ([Dewey et al., 2012; Farg et al., 2012](#)). Mutations in the angiogenin gene, ANG, a regulator of transcription, have also been linked to ALS ([McLaughlin et al., 2010](#)).

The loss of function of ataxin-1 (ATXN1), a regulator of RNA processing, was found to be related to the development of both sporadic and familial ALS. Misfolded ATXN1 also may form insoluble aggregates in diseased nuclei to disrupt transcription ([Morello et al., 2018](#)).

Also, mSOD1 directly contributes to a VEGFA deficiency, thereby reducing its neuroprotective function ([Dadon-Nachum et al., 2011](#)).

## II. Human disease pathways



**FIG. 4** Pathway 2: Mutations in SOD1 and associated proteins cause motor neuron death in familial ALS.

## References

- Disease number #105400, #205100, #608030, #613954, #611895, #602433 and others in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code G12.21. Diseases of the nervous system (G00-G99). (ICD-10, <https://icdlist.com>).
- Boillée, S., Vande Velde, C., Cleveland, D.W., 2006. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 52, 39–59. <https://doi.org/10.1016/j.neuron.2006.09.018>.
- Cozzolino, M., Ferri, A., Valle, C., Carri, M.T., 2013. Mitochondria and ALS: implications from novel genes and pathways. *Mol. Cell. Neurosci.* 55, 44–49. <https://doi.org/10.1016/j.mcn.2012.06.001>.
- Dadon-Nachum, M., Melamed, E., Offen, D., 2011. The “dying-back” phenomenon of motor neurons in ALS. *J. Mol. Neurosci.* 43, 470–477. <https://doi.org/10.1007/s12031-010-9467-1>.
- Desseille, C., Deforges, S., Biondi, O., Houdebine, L., D'amico, D., Lamazière, A., Caradeuc, C., Bertho, G., Bruneteau, G., Weill, L., Bastin, J., Djouadi, F., Salachas, F., Lopes, P., Chanoine, C., Massaad, C., Charbonnier, F., 2017. Specific physical exercise improves energetic metabolism in the skeletal muscle of amyotrophic-lateral-sclerosis mice. *Front. Mol. Neurosci.* 10. <https://doi.org/10.3389/fnmol.2017.00332>.
- Dewey, C.M., Cenik, B., Sephton, C.F., Johnson, B.A., Herz, J., Yu, G., 2012. TDP-43 aggregation in neurodegeneration: are stress granules the key? *Brain Res.* 1462, 16–25. <https://doi.org/10.1016/j.brainres.2012.02.032>.
- Farg, M.A., Soo, K.Y., Walker, A.K., Pham, H., Orian, J., Horne, M.K., Warraich, S.T., Williams, K.L., Blair, I.P., Atkin, J.D., 2012. Mutant FUS induces endoplasmic reticulum stress in amyotrophic lateral sclerosis and interacts with protein disulfide-isomerase. *Neurobiol. Aging* 33, 2855–2868. <https://doi.org/10.1016/j.neurobiolaging.2012.02.009>.
- Ferraiuolo, L., Kirby, J., Grierson, A.J., Sendtner, M., Shaw, P.J., 2011. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 7, 616–630. <https://doi.org/10.1038/nrneurol.2011.152>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Foran, E., Trott, D., 2009. Glutamate transporters and the excitotoxic path to motor neuron degeneration in amyotrophic lateral sclerosis. *Antioxid. Redox Signal.* 11, 1587–1602. <https://doi.org/10.1089/ars.2009.2444>.
- McLaughlin, R.L., Phukan, J., McCormack, W., Lynch, D.S., Greenway, M., Cronin, S., Saunders, J., Slowik, A., Tomik, B., Andersen, P.M., Bradley, D.G., Jakeman, P., Hardiman, O., 2010. Angiogenin levels and ANG genotypes: dysregulation in amyotrophic lateral sclerosis. *PLoS One* 5, e15402. <https://doi.org/10.1371/journal.pone.0015402>.
- Morello, G., Guaraccia, M., Spampinato, A.G., La Cognata, V., D'Agata, V., Cavallaro, S., 2018. Copy number variations in amyotrophic lateral sclerosis: piecing the mosaic tiles together through a systems biology approach. *Mol. Neurobiol.* 55, 1299–1322. <https://doi.org/10.1007/s12035-017-0393-x>.
- Pasinelli, P., Brown, R.H., 2006. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat. Rev. Neurosci.* 7, 710–723. <https://doi.org/10.1038/nrn1971>.
- Paul, P., Murphy, T., Oseni, Z., Sivalokanathan, S., de Belleroche, J.S., 2014. Pathogenic effects of amyotrophic lateral sclerosis-linked mutation in D-amino acid oxidase are mediated by D-serine. *Neurobiol. Aging* 35, 876–885. <https://doi.org/10.1016/j.neurobiolaging.2013.09.005>.

- Redler, R.L., Dokholyan, N.V., 2012. The complex molecular biology of amyotrophic lateral sclerosis (ALS). *Prog. Mol. Biol. Transl. Sci.* 107, 215–262. <https://doi.org/10.1016/B978-0-12-385883-2.00002-3>.
- Rosenblum, L.T., Shamamandri-Markandaiah, S., Ghosh, B., Foran, E., Lepore, A.C., Pasinelli, P., Trott, D., 2017. Mutation of the caspase-3 cleavage site in the astroglial glutamate transporter EAAT2 delays disease progression and extends lifespan in the SOD1-G93A mouse model of ALS. *Exp. Neurol.* 292, 145–153. <https://doi.org/10.1016/j.expneurol.2017.03.014>.
- Sheerin, U.-M., Schneider, S.A., Carr, L., Deuschl, G., Hopfner, F., Stamelou, M., Wood, N.W., Bhatia, K.P., 2014. ALS2 mutations: juvenile amyotrophic lateral sclerosis and generalized dystonia. *Neurology* 82, 1065–1067. <https://doi.org/10.1212/WNL.000000000000254>.
- Shi, P., Gal, J., Kwinter, D.M., Liu, X., Zhu, H., 2010. Mitochondrial dysfunction in amyotrophic lateral sclerosis. *Biochim. Biophys. Acta* 1802, 45–51. <https://doi.org/10.1016/j.bbadiis.2009.08.012>.
- Swarup, V., Julien, J.-P., 2011. ALS pathogenesis: recent insights from genetics and mouse models. *Prog. Neuropsychopharmacol. Biol. Psychiatry, The Neurobiology of Neurodegenerative Disorder: From Basic to Clinical Research* 35, 363–369. <https://doi.org/10.1016/j.pnpbp.2010.08.006>.
- Vilarino-Güell, C., Wider, C., Soto-Ortolaza, A.I., Cobb, S.A., Kachergus, J.M., Keeling, B.H., Dachsel, J.C., Hulihan, M.M., Dickson, D.W., Wszolek, Z.K., Uitti, R.J., Graff-Radford, N.R., Boeve, B.F., Josephs, K.A., Miller, B., Boylan, K.B., Gwinn, K., Adler, C.H., Aasly, J.O., Hentati, F., Destée, A., Krygowska-Wajs, A., Chartier-Harlin, M.-C., Ross, O.A., Rademakers, R., Farrer, M.J., 2009. Characterization of DCTN1 genetic variability in neurodegeneration. *Neurology* 72, 2024–2028. <https://doi.org/10.1212/WNL.0b013e3181a92c4c>.
- Webster, C.P., Smith, E.F., Shaw, P.J., De Vos, K.J., 2017. Protein homeostasis in amyotrophic lateral sclerosis: therapeutic opportunities? *Front. Mol. Neurosci.* 10, 123. <https://doi.org/10.3389/fnmol.2017.00123>.
- Yang, Y., Gozen, O., Watkins, A., Lorenzini, I., Lepore, A., Gao, Y., Vidensky, S., Brennan, J., Poulsen, D., Won Park, J., Li Jeon, N., Robinson, M.B., Rothstein, J.D., 2009. Presynaptic regulation of astroglial excitatory neurotransmitter transporter GLT1. *Neuron* 61, 880–894. <https://doi.org/10.1016/j.neuron.2009.02.010>.

## CHAPTER

## 5.3

## Familial hemiplegic migraine (FHM)

A familial hemiplegic migraine (FHM) is a form of migraine headache that runs in families. Migraines are characterized by intense, throbbing pain in one side of the head, and it is frequently accompanied by nausea and extreme sensitivity to light and sound. Each headache may last from a few hours to a few days.

Migraine headaches are recurrent headaches that are either preceded by a focal neurologic symptom (migraine with aura), occur independently without preceding focal neurologic symptoms (migraine without aura), or have atypical presentations (migraine variants). The migraine aura typically is characterized by visual or sensory symptoms that develop over 5 to 60 min. If aura includes motor weakness, the migraine is referred to as hemiplegic. (*Ferri and Ferri, 2018*).

Unusually, severe migraine episodes have been reported in some people with familial hemiplegic migraine. These episodes have included fever, seizures, prolonged weakness, coma, and, rarely, death (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Headaches can be triggered by certain foods, emotional stress, or minor head trauma.

Some patterns of neurological symptoms that precede a headache have been described. The most common symptoms associated with an aura include temporary visual changes such as blind spots (scotomas), flashing lights, zig-zagging lines, and double vision. An aura typically develops gradually over a few minutes and lasts about an hour.

After the initial symptoms of a given episode subside, long-lasting neurological symptoms, such as memory loss and problems with attention, often develop. Some patients (about 20%) also develop mild but enduring ataxia (lack of coordinated movement) and nystagmus (rapid involuntary eye movements).

Three forms of FHM have been identified. They are known as FHM1, FHM2, and FHM3 and are typically caused by mutations in the *CACNA1A*, *ATP1A2*, and *SCN1A* genes. Mutations in one of three genes (*SLC4A4*, *SLC1A3*, and *PRRT2*) are associated with sporadic hemiplegic migraine. Sporadic hemiplegic migraine is a rare form of a hemiplegic migraine that

occurs in patients without a family history, and it is caused by sporadic genetic mutations (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

There is a theory that high levels of glutamate in the brain lead to increased synthesis of nitric oxide (NO), which then induces the vasodilation of smooth muscle cells leading to the characteristic headache.

**Pathway 1.** *Glutamate overload enforced prostaglandins and NO synthesis in neurons.*

**Fig. 5** NO and PGH<sub>2</sub> synthesis in neuronal tissue.

**Fig. 6** NO-mediated vasodilation.

Genetic mutations linked to FHM affect different aspects of neuron function and may result in excess glutamate in the synaptic cleft:

**Pathway 2.** *Glutamate overload caused by mutations in the genes associated with familial and sporadic hemiplegic migraine (Fig. 7).*

## Key cellular contributors and processes

### Aura

#### Disease

The term migraine aura refers to a pattern of neurological symptoms that precede a headache. The most common symptoms associated with an aura are temporary visual changes such as blind spots, flashing lights, zig-zagging lines, and double vision. An aura usually develops gradually over a few minutes and lasts for up to an hour.

### Cortical spreading depression

#### Process

Cortical spreading depression (CSD) is a wave of slowly propagating excitation (depolarization) of brain cells followed by the inhibition of neuronal activity. CSD has been implicated in the pathophysiology of migraine.

### Dura mater

#### Anatomic structure

The dura mater is the outermost and toughest of the three sheaths covering the central nervous system (the brain and spinal cord). Made up of connective tissue, the dura mater contains two layers: the outer layer, which is rich in blood vessels, and the inner layer. There are large channels, known as dural venous sinuses, located between the two layers.

### Synaptic cleft

#### Anatomic structure

A synaptic cleft, characteristic of a chemical synapse, is the space between a neuron and its target cell, and it is where the release of neurotransmitters occurs.

### Vasoconstriction

#### Process

Vasoconstriction is the narrowing of blood vessels caused by the contraction of smooth muscles in their walls. Vasoconstriction decreases the blood flow through the vessels and increases blood pressure.

### Vasodilation

#### Process

Vasodilation is the widening of blood vessels caused by the relaxation of smooth muscle cells in their walls. Vasodilation increases the blood flow through the vessels and decreases blood pressure.

## Pathway 1

### Glutamate overload effects on the activation of prostaglandin E2 and NO synthesis

#### Incoming signals

High levels of glutamate in the synaptic cleft are found in each type of a hemiplegic migraine (familial and sporadic) (Vikelis and Mitsikostas, 2007). Excess glutamate triggers the synthesis of high levels of prostaglandin E2 (PGE2) and nitric oxide (NO) by both neurons and astrocytes in migraines. Increased amounts of PGE2 were detected in patients prior to and during a migraine attack.

Synthesized in neurons and astrocytes, PGE2 and NO in turn induce the synthesis of NO in endothelial cells and smooth muscle cells in dura mater. Increased NO levels cause vasodilation in the dura mater covering the brain and spinal cord (located most superficially). As connective tissue, the outer surface of the dura mater is rich in blood vessels.

#### Outcome effects

The main hypothesis explaining the origin of pain in hemiplegic migraine is associated with vasodilation. Glutamate can lead to vasodilation in a migraine by stimulating prostaglandin H2 (PGH2) and NO synthesis in neurons, astrocytes, endothelial, and smooth muscle cells of the dura mater leading to vasodilation and the consequent headache. It is believed that migraine pain is caused by the expansion of blood vessels. However, the exact mechanism underlying the development of pain in hemiplegic migraine is not completely understood (Antonova et al., 2013; Attwell et al., 2010; van der Kuy and Lohman, 2003).

Glutamate overload may also cause neuron apoptosis (see Amyotrophic Lateral Sclerosis, Pathway 1).

In addition, the overproduction of eicosanoids (arachidonic acid and prostaglandins) can explain fever—one symptom of hemiplegic migraine.

#### Signaling

##### **NO and PGH2 synthesis in neuronal tissue (Fig. 5)**

In patients with FHM glutamate accumulates in the synaptic cleft due to either genetic mutations (see Pathway 2) or other unclear reasons. The synaptic cleft is part of the synapse, the space between the presynaptic and postsynaptic membranes, where neurotransmitters are released.

In postsynaptic neurons, glutamate activates the membrane bound quisqualic acid receptors (AMPA-R), the N-methyl-D-aspartate (NMDA)

selective glutamate receptor, and voltage-gated  $\text{Ca}^{2+}$  channels, which together pump  $\text{Ca}^{2+}$  into the cell.  $\text{Ca}^{2+}$ -dependent signaling in neurons stimulates a specific phospholipase A2, group IVA (PLA2G4A), through the activation of protein kinase C, gamma (PRKCG), and calmodulin. Phospholipase A2 promotes the synthesis of arachidonic acid.

Glutamate also stimulates signaling of the metabotropic receptors (GRM5 or mGluR) on membranes of astrocytes. GRM5 signaling via coupled G proteins also activates phospholipase A2, which promotes the synthesis of arachidonic acid.

Arachidonic acid is a substrate for the synthesis of the prostaglandins H2 and E2 (PGH2 and PGE2). PGH2 is synthesized from arachidonic acid by prostaglandin synthase 1 (PTGS1) in astrocytes and by prostaglandin synthase 2 (PTGS2) in neurons. Prostaglandin E synthase (PTGES) produces PGE2.

NO synthesis in postsynaptic neurons depends on calcium signaling and the activation of NF- $\kappa$ B, which in turn activates transcription of the nitric oxide (NO) synthase genes neuronal NO synthase 1 (NOS1) and inducible NO synthase 2 (NOS2). In addition, calmodulin phosphorylates NOS1 directly to inactivate it ([van der Kuy and Lohman, 2003](#)).

### **NO synthesis in connective tissue (Fig. 6)**

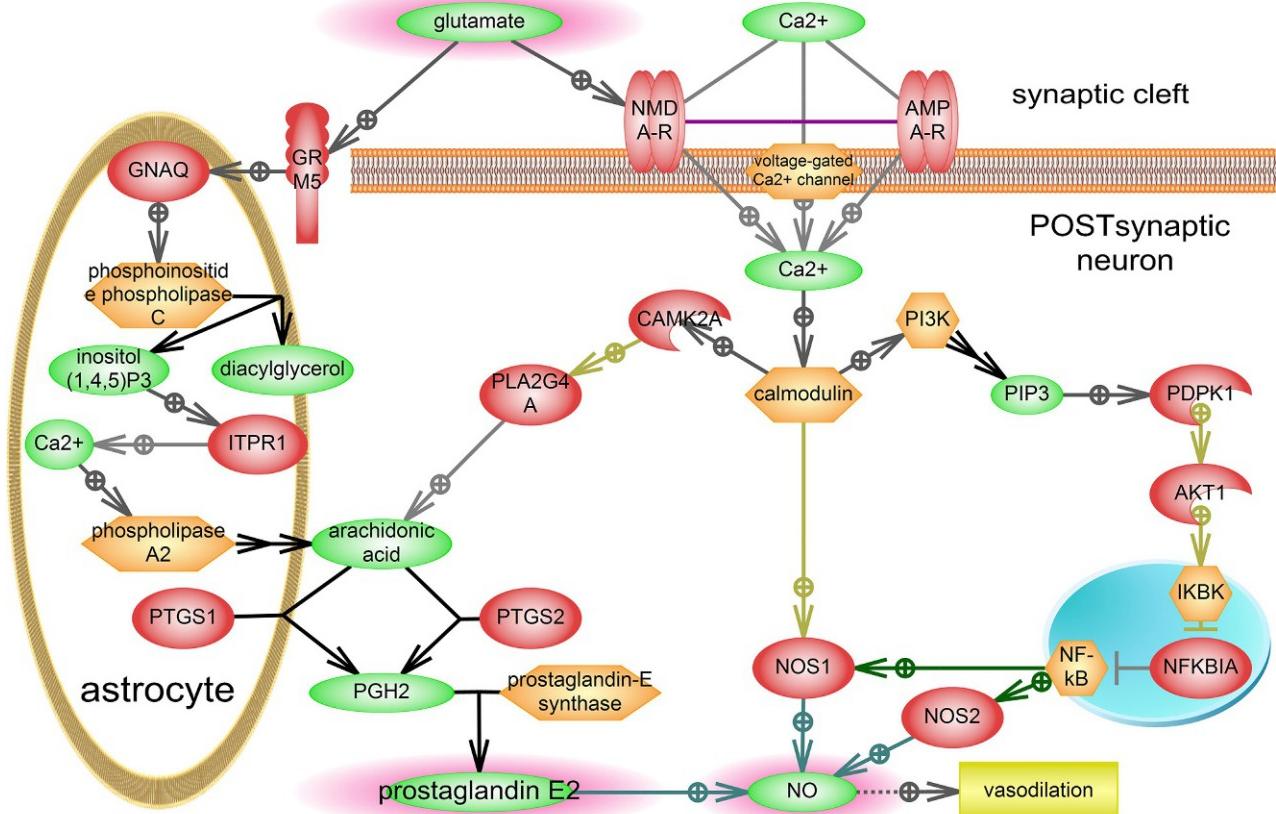
PGE2 interacts with the prostaglandin E2 receptor (PTGER2) on endothelial cell membranes. PTGER2 signaling in turn initiates the production of cAMP through G protein (GNAS) signaling. cAMP activates protein kinase A that itself phosphorylates and activates nitric oxide (NO) synthase 3 (NOS3).

Intracellular calcium can also trigger activation of the transient receptor potential cation channel (TRPV1) that in turn leads to phosphorylation of NOS3 by the calcium-/calmodulin-dependent protein kinase (CAMK2A).

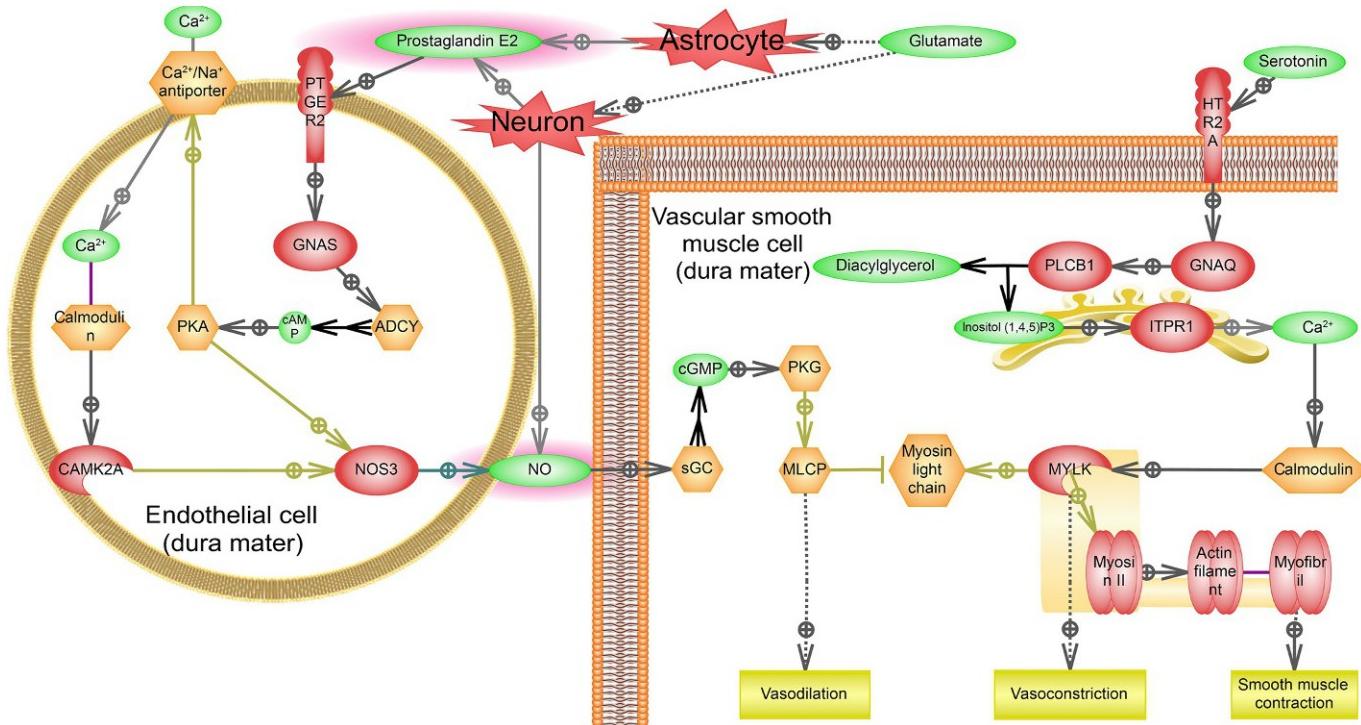
### **NO-mediated vasodilation (Fig. 6)**

The NO produced in epithelial cells flows into adjacent smooth muscle cells to activate the soluble guanylate cyclase (sGC)-dependent activation of protein kinase G (PKG). Protein kinase G in turn phosphorylates the myosin light-chain phosphatase (MLCP), which is part of the actin-myosin cytoskeleton in muscle cells. MLCP dephosphorylates the light chains of myosin causing vascular muscle cell relaxation, that is, vasodilation, in the dura mater.

The effects of vasoconstriction induced by serotonin are also shown on Fig. 6. Serotonin, through its receptors (HTR2A), can return blood vessels to their normal state of tone. Activation of these receptors triggers calcium-dependent signaling, which results in the inhibition of MLCP, and leads to vascular muscle contraction, for example, vasoconstriction.



**FIG. 5** Pathway 1: Glutamate overload enforced prostaglandins and NO synthesis in neurons. NO and PGH2 synthesis in neuronal tissue.



**FIG. 6** Pathway 1: Glutamate overload enforced prostaglandins and NO synthesis in neurons. NO-mediated vasodilation.

## Pathway 2

### Glutamate overload caused by mutations in the genes associated with familial and sporadic hemiplegic migraine ([Fig. 7](#))

#### Incoming signals

Mutations in different genes were linked with different types of familial hemiplegic migraine (Barrett et al., 2008; Pietrobon, 2010; Russell and Ducros, 2011).

More than 60 known loss-of-function mutations in the calcium voltage-gated channel subunit alpha 1 A (*CACNA1A*) gene may trigger familial hemiplegic migraine form 1 (FHM1). *CACNA1A* normally modulates the release of excitatory neurotransmitters such as glutamate in neuromuscular synapses and in central synapses.

Over 50 loss-of-function mutations in the ATPase  $\text{Na}^+/\text{K}^+$  transporting subunit alpha 2 (*ATP1A2*) gene have been associated with FHM2. Mutated *ATP1A2* leads to the accumulation of potassium ( $\text{K}^+$ ) in the extracellular space and sodium ( $\text{Na}^+$ ) within the cell.

FHM3 is linked to mutated sodium voltage-gated channel alpha subunit 1 (*SCN1A*) genes. To date, five mutations from five different families have been identified in the *SCN1A* gene. Mutated *SCN1A* causes increased levels of intracellular sodium ( $\text{Na}^+$ ) and elevates neuronal excitability.

In addition, mutations in *SLC1A3*, *PRRT2*, and *SLC4A4* have been strongly associated with sporadic hemiplegic migraine.

#### Outcome effects

Known mutations associated with FHM are the basis of glutamate and  $\text{K}^+$  accumulation in the synapse.

Excess glutamate leads to the release of increased levels of  $\text{K}^+$  in the synaptic cleft.

Excess  $\text{K}^+$  results in postsynaptic membrane depolarization, which plays an important role in the progression of cortical depression (spreading depolarization, cortical spreading depression, CSD). CSD characterized by fluctuating depolarization or moments of electrophysiological neuronal hyperactivity followed by a period of deceleration, which spread across the cortex at a velocity of 2–5 mm/min. The aura, which precedes migraine attacks, is a consequence of cortical spreading depression.

Also, the release of  $\text{K}^+$  causes constriction of proximal blood vessels in the brain. This type of vasoconstriction occurs simultaneously with cortical spreading depression and provokes the subsequent vasodilation and its associated headache.

Epilepsy and ataxia are common symptoms in patients with hemiplegic migraine, which may also be explained by abnormal glutamatergic transmission and CNS excitation.

## Signaling

CACNA1A encodes a subunit of the P/Q voltage-dependent calcium ( $\text{Ca}^{2+}$ ) channel. Different mutations in the CACNA1A gene cause different variants of ion channel malfunction such as conductivity disturbance, structural alterations, or shifted kinetics. Altered  $\text{Ca}^{2+}$  channels open at a lower voltage compared with wild type so that ion flow into the cell after membrane depolarization occurs more slowly. Also, the opening time of altered ion channels is prolonged. Therefore the overall level of  $\text{Ca}^{2+}$  entering the postsynaptic cell increases.  $\text{Ca}^{2+}$  stimulates the release of neurotransmitters (primarily glutamate) into the synaptic cleft from both glia and presynaptic neurons (Cao et al., 2004; Klimov, 2017; Russell and Ducros, 2011; Tottene et al., 2002).

SCN1A encodes the pore-forming alpha-1-subunit of the voltage-dependent sodium ( $\text{Na}^+$ ) channel in presynaptic neurons. This type of ion channel is present mainly in the body and proximal dendrites of inhibitory neurons. Mutated SCN1A causes an increase of intracellular  $\text{Na}^+$ , which in turn stimulates the release of glutamate into the synaptic cleft (Dichgans et al., 2005; Russell and Ducros, 2011).

The loss of function of the proline-rich transmembrane protein 2 (PRRT2) is probably important for regulating the spontaneous synaptic vesicle-mediated exocytosis of glutamate (glutamate release). The spontaneous exocytosis of glutamate usually occurs when the volume of vesicles with glutamate grows. The exact molecular mechanism of the action of mutated PRRT2 is not well known (Castiglioni et al., 2013; Dale et al., 2012; Marini et al., 2012; Russell and Ducros, 2011).

Excess glutamate can be removed by astrocytes, which in turn convert it to glutamine. Glutamine is then transferred into neurons where it is converted back to glutamate (Vikelis and Mitsikostas, 2007).

The solute carrier family 1 member 3 (SLC1A3) gene encodes a high-affinity glutamate transporter expressed on the surface of astrocytes that is responsible for the uptake of glutamate from the synaptic cleft. Known mutations in the SLC1A3 gene lead to excessive levels of glutamate in the synaptic cleft (de Vries et al., 2009; Jen et al., 2005; Russell and Ducros, 2011). (Also see Amyotrophic Lateral Sclerosis, Pathway 1.)

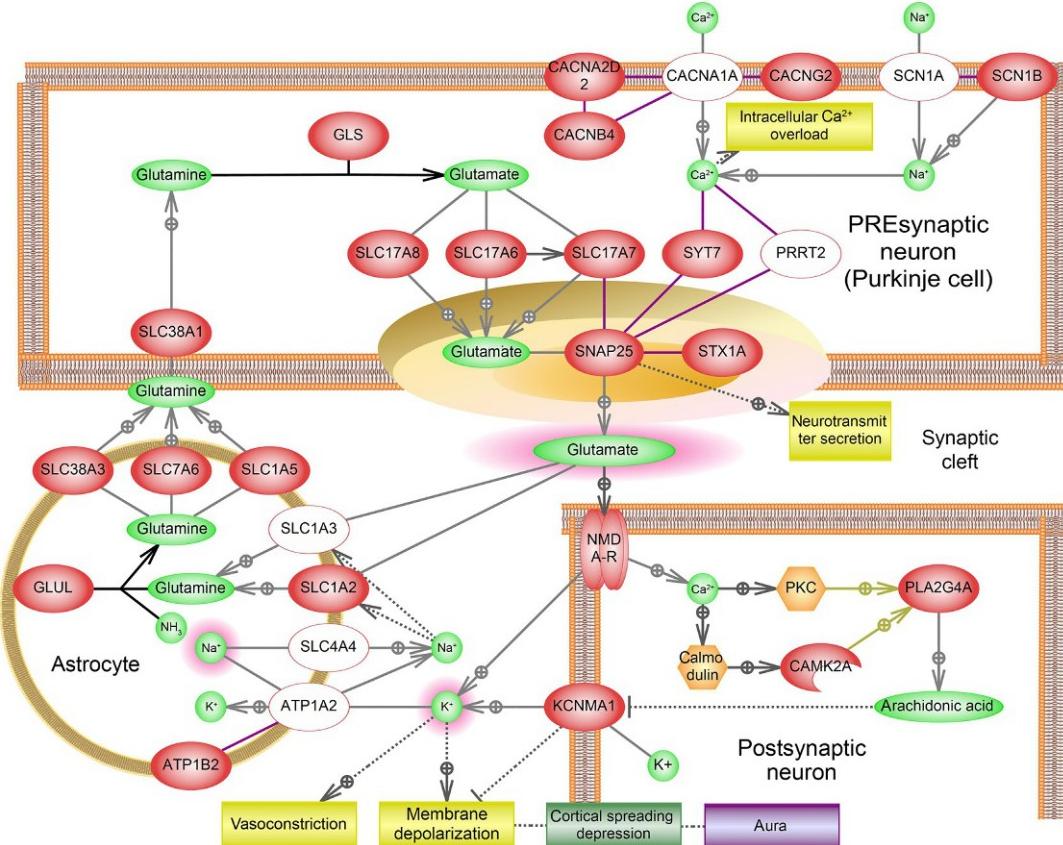
For correct glutamate reuptake by astrocytes, the glial glutamate transporters (SLC1A3 and SLC1A2) need  $\text{Na}^+$  ions in order to transport glutamate into the cell.

ATP1A2 encodes the alpha-2 subunit of neuronal and the glial  $\text{Na}/\text{K}$ -ATPase. This ATPase is a transmembrane ion pump, which contributes

to membrane potential regulation by pumping potassium ( $K^+$ ) into glial cells and sodium ( $Na^+$ ) out of glial cells. The reduced activity of mutated ATP1A2 leads to disrupted ion balance, thereby resulting in the ineffective reuptake of glutamate from the synaptic cleft by glial cells. A deletion (del65bp) in the SLC4A4 gene mimics mutations in the ATP1A2 gene. When mutated SLC4A4 is expressed in astrocytes, glutamate reuptake from the synaptic cleft is decreased. This disrupts the function of glutamate transporters and increases the glutamate concentration in the synaptic cleft (Russell and Ducros, 2011; Suzuki et al., 2010).

Excess glutamate in the synaptic cleft activates the ionotropic N-methyl-D-aspartate receptor (NMDA-R) on postsynaptic neurons. Activation of NMDA-R signaling leads to the release of potassium ( $K^+$ ) ions from postsynaptic neurons and in turn leads to membrane depolarization (another aspect of NMDA-R signaling, see in Amyotrophic Lateral Sclerosis, Pathway 1) (De Fusco et al., 2003; Russell and Ducros, 2011; Segall et al., 2004).

There are feedback mechanisms, which decrease neuronal membrane polarization. For example,  $K^+$  can be removed from the extracellular space by the calcium-activated potassium channel (KCNMA1), which is in turn activated by the NMDA-dependent cytosolic  $Ca^{2+}$ -dependent phospholipase A2 (PLA2G4A).



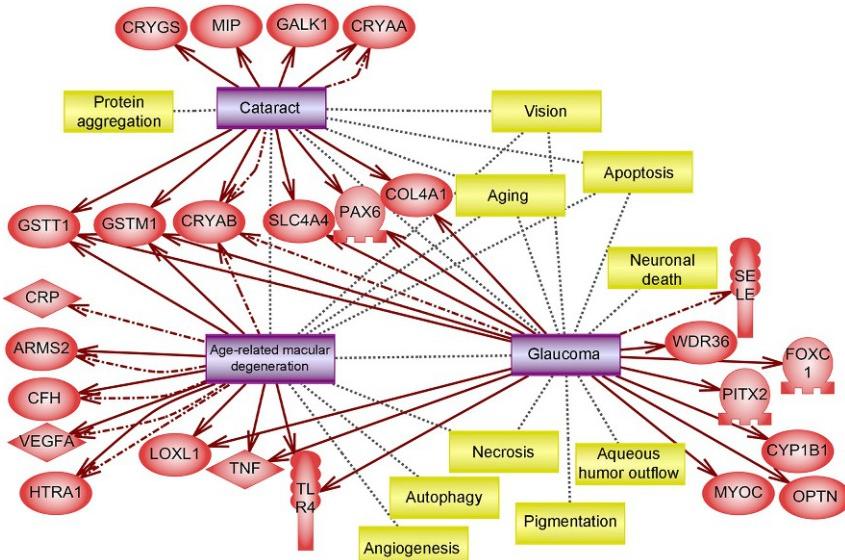
**FIG. 7** Pathway 2: Glutamate overload caused by mutations in the genes associated with familial and sporadic hemiplegic migraine.

## References

- Disease number FHM1 (OMIM:#141500), FHM2 (OMIM: #602481), *SCN1A* gene (OMIM: #609634) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)). ICD-10: disease code G12.21. Diseases of the nervous system (G00-G99). (ICD-10, <https://icdlist.com>).
- Antonova, M., Wienecke, T., Olesen, J., Ashina, M., 2013. Prostaglandins in migraine: update. *Curr. Opin. Neurol.* 26, 269–275. <https://doi.org/10.1097/WCO.0b013e328360864b>.
- Attwell, D., Buchan, A.M., Charpak, S., Lauritzen, M., Macvicar, B.A., Newman, E.A., 2010. Glial and neuronal control of brain blood flow. *Nature* 468, 232–243. <https://doi.org/10.1038/nature09613>.
- Barrett, C.F., van den Maagdenberg, A.M.J.M., Frants, R.R., Ferrari, M.D., 2008. Familial hemiplegic migraine. *Adv. Genet.* 63, 57–83. [https://doi.org/10.1016/S0065-2660\(08\)01003-1](https://doi.org/10.1016/S0065-2660(08)01003-1).
- Cao, Y.-Q., Piedras-Rentería, E.S., Smith, G.B., Chen, G., Harata, N.C., Tsien, R.W., 2004. Presynaptic  $\text{Ca}^{2+}$  channels compete for channel type-preferring slots in altered neurotransmission arising from  $\text{Ca}^{2+}$  channelopathy. *Neuron* 43, 387–400. <https://doi.org/10.1016/j.neuron.2004.07.014>.
- Castiglioni, C., López, I., Riant, F., Bertini, E., Terracciano, A., 2013. PRRT2 mutation causes paroxysmal kinesigenic dyskinesia and hemiplegic migraine in monozygotic twins. *Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc.* 17, 254–258. <https://doi.org/10.1016/j.ejpn.2012.10.010>.
- Dale, R.C., Gardiner, A., Antony, J., Houlden, H., 2012. Familial PRRT2 mutation with heterogeneous paroxysmal disorders including paroxysmal torticollis and hemiplegic migraine. *Dev. Med. Child Neurol.* 54, 958–960. <https://doi.org/10.1111/j.1469-8749.2012.04394.x>.
- De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., Ballabio, A., Aridon, P., Casari, G., 2003. Haploinsufficiency of ATP1A2 encoding the  $\text{Na}^+/\text{K}^+$  pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat. Genet.* 33, 192–196. <https://doi.org/10.1038/ng1081>.
- de Vries, B., Mamsa, H., Stam, A.H., Wan, J., Bakker, S.L.M., Vanmolkot, K.R.J., Haan, J., Terwindt, G.M., Boon, E.M.J., Howard, B.D., Frants, R.R., Baloh, R.W., Ferrari, M.D., Jen, J.C., van den Maagdenberg, A.M.J.M., 2009. Episodic ataxia associated with EAAT1 mutation C186S affecting glutamate reuptake. *Arch. Neurol.* 66, 97–101. <https://doi.org/10.1001/archneurol.2008.535>.
- Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., Ferrari, M.D., Herzog, J., van den Maagdenberg, A.M.J.M., Pusch, M., Strom, T.M., 2005. Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. *Lancet* 366, 371–377. [https://doi.org/10.1016/S0140-6736\(05\)66786-4](https://doi.org/10.1016/S0140-6736(05)66786-4).
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Jen, J.C., Wan, J., Palos, T.P., Howard, B.D., Baloh, R.W., 2005. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. *Neurology* 65, 529–534. <https://doi.org/10.1212/01.wnl.0000172638.58172.5a>.
- Klimov, E., 2017. Familial hemiplegic migraine type I: the molecular signaling pathway. *J. Neurol. Stroke* 7. <https://doi.org/10.15406/jnsk.2017.07.00249>.
- Marini, C., Conti, V., Mei, D., Battaglia, D., Lettori, D., Losito, E., Bruccini, G., Tortorella, G., Guerrini, R., 2012. PRRT2 mutations in familial infantile seizures, paroxysmal dyskinesia, and hemiplegic migraine. *Neurology* 79, 2109–2114. <https://doi.org/10.1212/WNL.0b013e3182752ca2>.
- Pietrobon, D., 2010. Biological science of headache channels. *Handb. Clin. Neurol.* 97, 73–83. [https://doi.org/10.1016/S0072-9752\(10\)97005-X](https://doi.org/10.1016/S0072-9752(10)97005-X).
- Russell, M.B., Ducros, A., 2011. Sporadic and familial hemiplegic migraine: pathophysiological mechanisms, clinical characteristics, diagnosis, and management. *Lancet Neurol.* 10, 457–470. [https://doi.org/10.1016/S1474-4422\(11\)70048-5](https://doi.org/10.1016/S1474-4422(11)70048-5).

- Segall, L., Scanzano, R., Kaunisto, M.A., Wessman, M., Palotie, A., Gargus, J.J., Blostein, R., 2004. Kinetic alterations due to a missense mutation in the Na,K-ATPase alpha2 subunit cause familial hemiplegic migraine type 2. *J. Biol. Chem.* 279, 43692–43696. <https://doi.org/10.1074/jbc.M407471200>.
- Suzuki, M., Van Paesschen, W., Stalmans, I., Horita, S., Yamada, H., Bergmans, B.A., Legius, E., Riant, F., De Jonghe, P., Li, Y., Sekine, T., Igarashi, T., Fujimoto, I., Mikoshiba, K., Shimadzu, M., Shiohara, M., Braverman, N., Al-Gazali, L., Fujita, T., Seki, G., 2010. Defective membrane expression of the Na(+)–HCO<sub>3</sub>(-) cotransporter NBCe1 is associated with familial migraine. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15963–15968. <https://doi.org/10.1073/pnas.1008705107>.
- Tottene, A., Fellin, T., Pagnutti, S., Luvisetto, S., Striessnig, J., Fletcher, C., Pietrobon, D., 2002. Familial hemiplegic migraine mutations increase Ca(2+) influx through single human CaV2.1 channels and decrease maximal CaV2.1 current density in neurons. *Proc. Natl. Acad. Sci. U. S. A.* 99, 13284–13289. <https://doi.org/10.1073/pnas.192242399>.
- van der Kuy, P.-H.M., Lohman, J.J., 2003. The role of nitric oxide in vascular headache. *Pharm. World Sci.* 25, 146–151.
- Vikelis, M., Mitsikostas, D.D., 2007. The role of glutamate and its receptors in migraine. *CNS Neurol. Disord. Drug Targets* 6, 251–257.

# Diseases of the eye



## OUTLINE

<b>Glaucoma</b>	<b>261</b>
<b>Age-related macular degeneration</b>	<b>273</b>
<b>Cataract</b>	<b>285</b>

The comprehensive list of various eye disorders includes congenital malformations, eye infections, and other acquired pathologies. If inherited and infectious diseases are excluded from the list, the most common causes of blindness are glaucoma, age-related macular degeneration, cataract, and diabetic retinopathy. Among these, cataract is the one most

easily diagnosed and treatable by surgery. Diabetic retinopathy is the late complication of diabetes mellitus. Early detection and the timely treatment of diabetes mellitus help prevent diabetic retinopathy. The following chapter focuses on glaucoma, cataract, and age-related macular degeneration as the most common eye disorders with no effective treatment yet.

Glaucoma along with cataract is the primary causes of blindness. Glaucoma is a group of disorders in which damage to the optic nerve causes vision loss. Open-angle glaucoma is the most common type of glaucoma, while closed-angle glaucoma and normal-tension glaucoma are less common.

Age-related macular degeneration (ARMD) is the fourth most common cause of blindness. ARMD may result in blurred or no vision in the center of the visual field. Often, during the initial stages of the disease, there are no symptoms. Over time, patients experience a gradual worsening of vision resulting in complete blindness or loss of central vision. The disease progression usually occurs in people over 50 years of age.

## CHAPTER

## 6.1

## Glaucoma

In most people with glaucoma, the damage to the optic nerves is caused by increased pressure within the eyes (intraocular pressure). Intraocular pressure depends on a balance between fluid entering and leaving the eyes.

Glaucoma is a chronic degenerative optic neuropathy in which the neuro-retinal rim of the optic nerve becomes progressively thinner, thereby enlarging the optic-nerve cup. (*Ferri and Ferri, 2018*).

The classification of glaucoma is based on the appearance of the irido-corneal angle (i.e., open angle vs closed angle) and is further subdivided into primary and secondary types. Primary open-angle glaucoma can occur with or without elevated intraocular pressure (IOP). Normal-tension glaucoma refers to primary open-angle glaucoma without elevated intraocular pressure (*Ferri and Ferri, 2018*).

The mechanism of open-angle glaucoma is believed to depend on the slow exit of aqueous humor (AH) through the trabecular meshwork. In contrast, in closed-angle glaucoma, the iris blocks the trabecular meshwork.

A positive family history is a risk factor for glaucoma. Mutations in several genes including *MYOC*, *ASB10*, *WDR36*, *NTF4*, *TBK1*, and *RPGRIPI* are associated with primary open-angle glaucoma. Normal-tension glaucoma is associated with mutations of the *OPA1* and *OPTN* genes (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Obstruction of aqueous humor drainage through trabecular meshwork is often a significant feature of glaucoma:

**Pathway 1.** Trabecular meshwork and Schlemm's canal endothelial cell volume and contractility (Fig. 1).

Mutations in several genes (e.g., *MYOC*) may lead to the destruction of trabecular meshwork cells:

**Pathway 2.** *MYOC*-associated glaucoma (Fig. 2).

A hallmark of glaucoma is the damage to the optic nerve although the precise mechanism of this process is not clear. Neurotrophic factor deprivation is regarded as one of the main causes of retinal ganglion cell death:

**Pathway 3.** *Role of neurotrophic factor deprivation in retinal ganglion cell death (Fig. 3).*

## Key cellular contributors and processes

### Aqueous humor drainage

#### Process

Aqueous humor (AH) is a transparent liquid that occupies the space between the crystalline lens and the cornea of the eye. AH resembles plasma, but it contains lower protein and glucose concentrations. AH nourishes the cornea and the lens and is involved in intraocular pressure maintenance.

### Aqueous humor production and function

#### Process

Regulation of aqueous humor (AH) outflow is important for intraocular pressure maintenance. The drainage path for AH starts in the posterior chamber of the eye. Further, AH flows into the area between the posterior iris and the anterior lens and then through the pupil and enters the anterior chamber. From the anterior chamber, AH leaves the eye through the trabecular meshwork (TM) and flows into Schlemm's canal. Further, AH flows through the collector channels into the episcleral veins. The greatest resistance to AH outflow is contained at the TM.

### Intraocular pressure

#### Process

Intraocular pressure (IOP) is the intraocular fluid pressure inside the eyeball. IOP depends on the balance between the production and drainage of aqueous humor mainly through the trabecular meshwork. IOP is increased in glaucoma.

### Optic nerve

#### Anatomic structure

The optic nerve (cranial nerve II, CN II) is a paired nerve that conducts visual impulses from the retina to the brain. The optic nerve consists of retinal ganglion cell axons and glial cells. In glaucoma, the optic nerve is damaged due to increased intraocular pressure.

### Trabecular meshwork

#### Anatomic structure

The trabecular meshwork (TM) is an area in the anterior chamber of the eye lined by cells called trabeculocytes. The TM provides resistance to aqueous humor flow and is crucial for intraocular pressure maintenance.

## Pathway 1

### Trabecular meshwork and Schlemm's canal endothelial cell volume and contractility (Fig. 1)

#### Incoming signaling

The trabecular meshwork (TM) and Schlemm's canal comprise the outflow system for aqueous humor (AH) in the eye. TM is a network of beams covered with endothelial cells. Outflow resistance is dependent on the condition of the TM extracellular matrix and on the size and volume of the endothelial cells covering the constituent beams.

#### Outcome effects

Contraction of TM cells decreases the permeability of the TM due to the reduced size of the intercellular spaces, and it leads to an increase of intraocular pressure (IOP) that may provoke open-angle glaucoma. When TM cells relax, the opposite effect occurs: the permeability of the TM increases, and IOP decreases. Relaxation of TM cells is regarded as one of the primary therapeutic targets in the treatment of glaucoma.

#### Signaling

Endothelial cell nitric oxide synthase (NOS3) has been shown to be activated as a result of IOP increase to produce nitric oxide (NO). NO activates soluble guanylate cyclase (sGC), formation of cGMP, and activation of protein kinase G (PKG) with the subsequent phosphorylation of target proteins including BK channels (e.g., large conductance  $\text{Ca}^{2+}$ -activated potassium channel) and  $\text{K}:\text{Cl}^-$  symporters with subsequent potassium ( $\text{K}^+$ ) efflux. In addition, water efflux is mediated by aquaporin 1 (AQP1). As a result, cell volume decreases, thereby allowing easy outflow of AH leading to a decrease in IOP. TM is a contractile tissue itself with properties similar to smooth muscle. TM contractility is linked to the ras homolog gene family member A (RHOA) protein, which inhibits myosin light-chain phosphatase (MLCP), resulting in the accumulation of phosphorylated myosin light chain, which is then capable of interacting with actin to produce contraction. Contraction of TM cells decreases permeability of the TM due to the reduced size of the intercellular spaces. Similarly, when TM cells relax, the opposite effect appears; tissue permeability increases. Rho kinase (ROCK1) inhibitors are potentially a novel class of glaucoma therapeutics. In addition, elevated concentrations of endothelin-1 (EDN1) have been documented in the AH of patients with glaucoma. EDN1 is capable of inducing contraction of both TM cells and

the cellular matrix. Contraction seems to be triggered by the activation of the RHOA/ROCK1 pathway, which leads to an increase in outflow resistance and consequently to IOP elevation (Dismuke et al., 2008; Ellis, 2011; Ellis et al., 2010; Goel et al., 2010; Llobet et al., 2003; Rocha-Sousa et al., 2013; Verkman et al., 2008; Wang and Chang, 2014).

## II. Human disease pathways

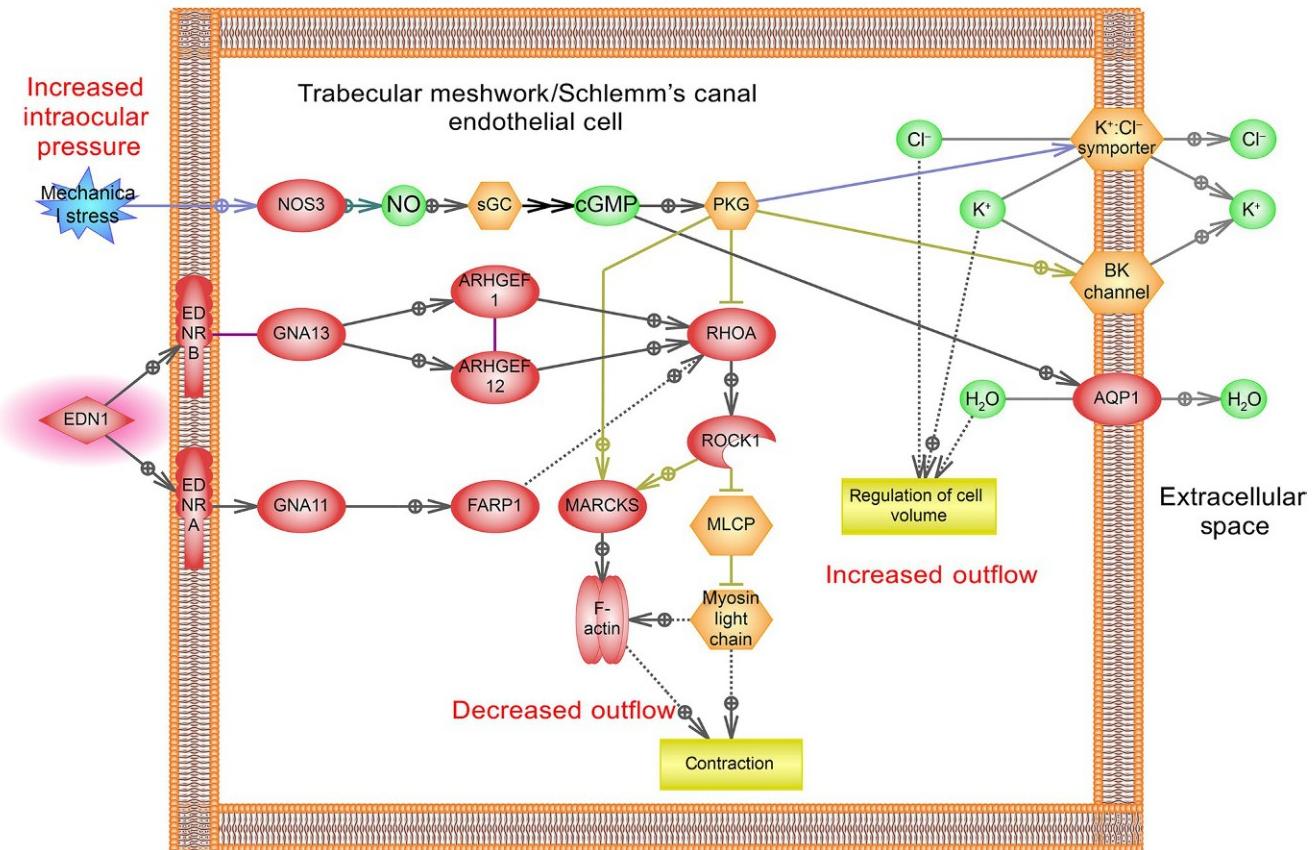


FIG. 1 Pathway 1: Trabecular meshwork and Schlemm's canal endothelial cell volume and contractility.

## Pathway 2

### MYOC-associated glaucoma (Fig. 2)

#### Incoming signaling

Approximately 4% of the cases of adult-onset primary open-angle glaucoma and 10%–33% of juvenile-onset cases are associated with myocilin (*MYOC*) mutations. *MYOC* is a secreted glycoprotein of unknown function. Most likely, mutations in *MYOC* have a pathogenic effect due to the inability of the protein to fold correctly.

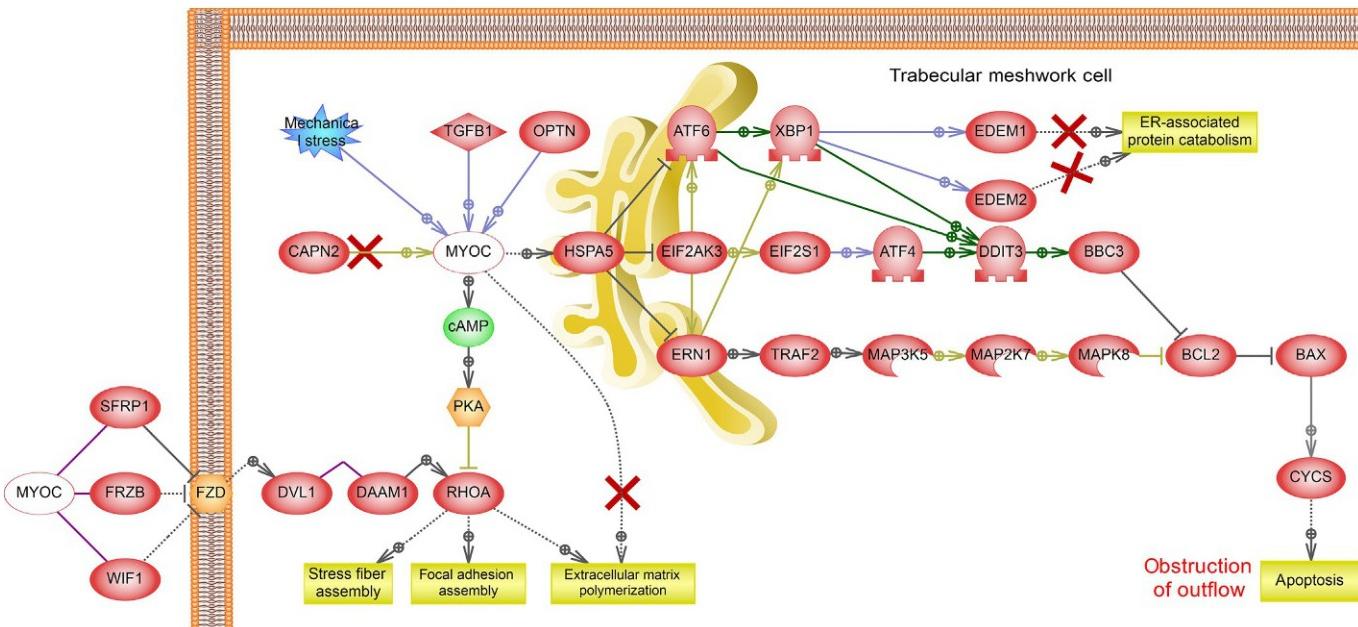
#### Outcome effects

The misfolded *MYOC* protein ultimately activates a mitochondria-independent apoptosis pathway, which causes cell death and the subsequent breakdown of the trabecular meshwork cell structure, obstruction of the aqueous humor outflow pathway, and consequent elevation of intraocular pressure.

#### Signaling

When misfolded, mutated *MYOC* forms aggregates in the endoplasmic reticulum (ER) and in the cytoplasm. The misfolded protein ultimately results in an unfolded protein response (UPR) and activates a mitochondria-independent apoptosis pathway. In addition, *MYOC* mutations greatly reduce the quantity of *MYOC* that is secreted into the AH, supporting the idea that failure to secrete *MYOC* is one of the central events in the pathogenesis of *MYOC*-associated glaucoma. Normally, *MYOC* is cleaved by calpain 2 (CAPN2) into two fragments: a 20-kDa N-terminal fragment and a 35-kDa C-terminal fragment. The 35-kDa fragment and full-length *MYOC* have been shown to be excreted into the extracellular matrix (ECM) and thus hypothesized to maintain a regular ECM structure. Mutations in *MYOC* disturb this cleavage and cause the breakdown of ECM structure in TM. *MYOC* affects TM adhesion properties through RHOA. *MYOC* also has been demonstrated to be a modulator of the WNT signaling pathway. *MYOC* interacts with WNT receptors of the Frizzled (FZD) family; WNT antagonists of the secreted FZD-related protein (SFRP1) family; and WNT inhibitory factor 1 (WIF1), which together modulate the organization of actin cytoskeleton and stimulate the formation of stress fibers, which are critical for the contractility of TM and therefore IOP regulation. Expression of *MYOC* may be induced by transforming growth factor beta 1 (TGFB1), optineurin (OPTN), and mechanical stress (Anholt and Carbone, 2013; Fuse, 2010; Gemenetzi et al., 2012; Jing et al., 2012; Jones and Rhee, 2006; Kwon et al., 2009; Menaa et al., 2011; Shen et al., 2008).

## II. Human disease pathways



**FIG. 2** Pathway 2: MYOC-associated glaucoma.

## Pathway 3

### Role of neurotrophic factor deprivation in retinal ganglion cell death ([Fig. 3](#))

#### Incoming signaling

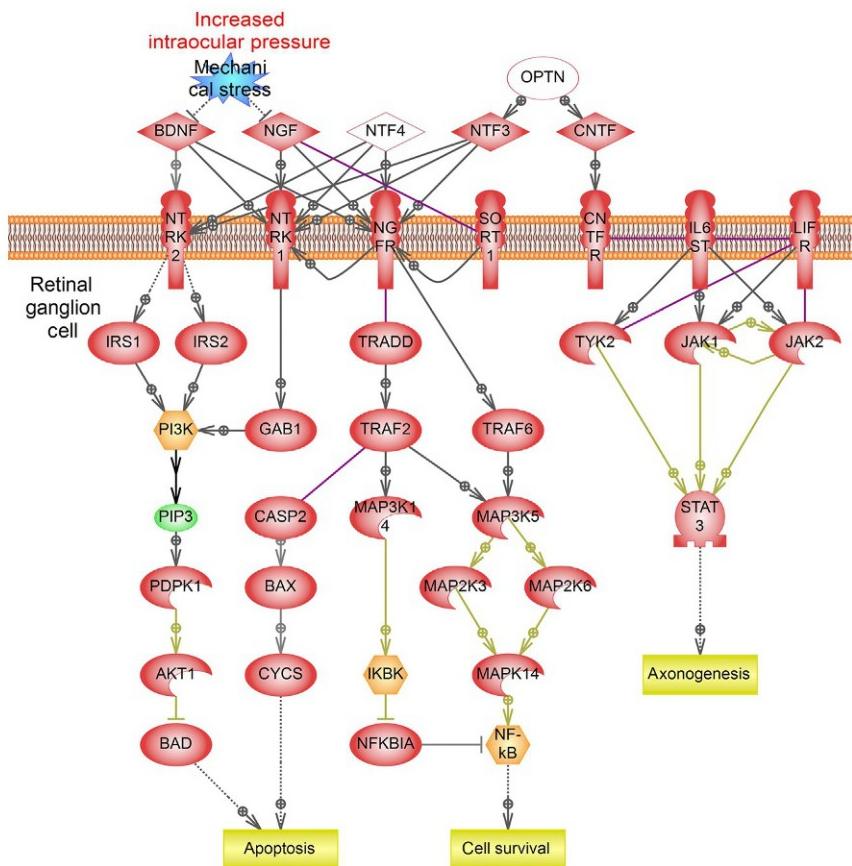
The presence of neurotrophic factors (NTFs) supports cell survival, while NTF deprivation may lead to apoptosis. A range of NTFs, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophins (NTF3 and NTF4), and ciliary neurotrophic factor (CNTF), are implicated in this process.

#### Outcome effects

The interruption of axonal transport and NTF deprivation due to elevated intraocular pressure are believed to cause the apoptotic death of retinal ganglion cells (RGCs).

#### Signaling

The selective activation of neurotrophic tyrosine kinase receptor type 1 (NTRK1) by NGF promotes survival of injured RGCs. The coexpression of nerve growth factor receptor (NGFR) and NTRK1 increases NGF binding and enhances NGF-mediated NTRK1 activation. However, when NTRK1/NGFR ratios are low or when neurotrophin levels are limited, NGFR activation leads to neuronal death. Pro-NGF may initiate cell apoptosis by binding to the NGFR-SORT1 (sortilin 1) receptor complex. In addition, CNTF has been shown to stimulate axonal regeneration via IL6ST/STAT3 signaling. In rare cases of glaucoma, NTF4 may be absent due to a mutation. The knockdown of endogenous OPTN induces apoptotic cell death due to the reduced secretion of NTF3 and CNTF. NTFs are regarded as potential therapeutic agents in glaucoma ([Almasieh et al., 2012](#); [Johnson et al., 2009](#); [Lambiase et al., 2011](#); [Leibinger et al., 2013](#); [Sippl et al., 2011](#); [Wen et al., 2012](#)).



**FIG. 3** Pathway 3: Role of neurotrophic factor deprivation in retinal ganglion cell death.

## References

- Disease number # 137750, # 137760, # 231300, # 606657 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code H40. Diseases of the eye and adnexa (H00-H59). (ICD-10, <https://icdlist.com>). ICD-11: disease code Q61.
- Almasieh, M., Wilson, A.M., Morquette, B., Cueva Vargas, J.L., Di Polo, A., 2012. The molecular basis of retinal ganglion cell death in glaucoma. *Prog. Retin. Eye Res.* 31, 152–181. <https://doi.org/10.1016/j.preteyeres.2011.11.002>.
- Anholt, R.R.H., Carbone, M.A., 2013. A molecular mechanism for glaucoma: endoplasmic reticulum stress and the unfolded protein response. *Trends Mol. Med.* 19, 586–593. <https://doi.org/10.1016/j.molmed.2013.06.005>.
- Dismuke, W.M., Mbadugha, C.C., Ellis, D.Z., 2008. NO-induced regulation of human trabecular meshwork cell volume and aqueous humor outflow facility involve the BKCa ion channel. *Am. J. Physiol. Cell Physiol.* 294, C1378–C1386. <https://doi.org/10.1152/ajpcell.00363.2007>.
- Ellis, D.Z., 2011. Guanylate cyclase activators, cell volume changes and IOP reduction. *Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol.* 28, 1145–1154. <https://doi.org/10.1159/000335866>.
- Ellis, D.Z., Sharif, N.A., Dismuke, W.M., 2010. Endogenous regulation of human Schlemm's canal cell volume by nitric oxide signaling. *Invest. Ophthalmol. Vis. Sci.* 51, 5817–5824. <https://doi.org/10.1167/iovs.09-5072>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fuse, N., 2010. Genetic bases for glaucoma. *Tohoku J. Exp. Med.* 221, 1–10.
- Gemenetzi, M., Yang, Y., Lotery, A.J., 2012. Current concepts on primary open-angle glaucoma genetics: a contribution to disease pathophysiology and future treatment. *Eye* 26, 355–369. <https://doi.org/10.1038/eye.2011.309>.
- Goel, M., Picciani, R.G., Lee, R.K., Bhattacharya, S.K., 2010. Aqueous humor dynamics: a review. *Open Ophthalmol. J.* 4, 52–59. <https://doi.org/10.2174/1874364101004010052>.
- Jing, G., Wang, J.J., Zhang, S.X., 2012. ER stress and apoptosis: a new mechanism for retinal cell death. *Exp. Diabetes Res.* 2012, 589589. <https://doi.org/10.1155/2012/589589>.
- Johnson, E.C., Guo, Y., Cepurna, W.O., Morrison, J.C., 2009. Neurotrophin roles in retinal ganglion cell survival: lessons from rat glaucoma models. *Exp. Eye Res.* 88, 808–815. <https://doi.org/10.1016/j.exer.2009.02.004>.
- Jones, R., Rhee, D.J., 2006. Corticosteroid-induced ocular hypertension and glaucoma: a brief review and update of the literature. *Curr. Opin. Ophthalmol.* 17, 163–167. <https://doi.org/10.1097/01.icu.0000193079.55240.18>.
- Kwon, H.-S., Lee, H.-S., Ji, Y., Rubin, J.S., Tomarev, S.I., 2009. Myocilin is a modulator of Wnt signaling. *Mol. Cell. Biol.* 29, 2139–2154. <https://doi.org/10.1128/MCB.01274-08>.
- Lambiase, A., Mantelli, F., Sacchetti, M., Rossi, S., Aloe, L., Bonini, S., 2011. Clinical applications of NGF in ocular diseases. *Arch. Ital. Biol.* 149, 283–292.
- Leibinger, M., Andreadaki, A., Diekmann, H., Fischer, D., 2013. Neuronal STAT3 activation is essential for CNTF- and inflammatory stimulation-induced CNS axon regeneration. *Cell Death Dis.* 4, e805. <https://doi.org/10.1038/cddis.2013.310>.
- Llobet, A., Gasull, X., Gual, A., 2003. Understanding trabecular meshwork physiology: a key to the control of intraocular pressure? *News Physiol. Sci. Int. J. Physiol. Prod. Jointly Int. Union Physiol. Sci. Am. Physiol. Soc.* 18, 205–209.
- Menaa, F., Braghini, C.A., Vasconcellos, J.P.C.D., Menaa, B., Costa, V.P., Figueiredo, E.S.D., Melo, M.B.D., 2011. Keeping an eye on myocilin: a complex molecule associated with primary open-angle glaucoma susceptibility. *Molecules* 16, 5402–5421. <https://doi.org/10.3390/molecules16075402>.

- Rocha-Sousa, A., Rodrigues-Araújo, J., Gouveia, P., Barbosa-Breda, J., Azevedo-Pinto, S., Pereira-Silva, P., Leite-Moreira, A., 2013. New therapeutic targets for intraocular pressure lowering. *ISRN Ophthalmol.* 2013, 261386.<https://doi.org/10.1155/2013/261386>.
- Shen, X., Koga, T., Park, B.-C., SundarRaj, N., Yue, B.Y.J.T., 2008. Rho GTPase and cAMP/protein kinase a signaling mediates myocilin-induced alterations in cultured human trabecular meshwork cells. *J. Biol. Chem.* 283, 603–612. <https://doi.org/10.1074/jbc.M708250200>.
- Sippl, C., Bosserhoff, A.K., Fischer, D., Tamm, E.R., 2011. Depletion of optineurin in RGC-5 cells derived from retinal neurons causes apoptosis and reduces the secretion of neurotrophins. *Exp. Eye Res.* 93, 669–680. <https://doi.org/10.1016/j.exer.2011.08.011>.
- Verkman, A.S., Ruiz-Ederra, J., Levin, M.H., 2008. Functions of aquaporins in the eye. *Prog. Retin. Eye Res.* 27, 420–433. <https://doi.org/10.1016/j.preteyeres.2008.04.001>.
- Wang, S.K., Chang, R.T., 2014. An emerging treatment option for glaucoma: rho kinase inhibitors. *Clin. Ophthalmol.* 8, 883–890. <https://doi.org/10.2147/OPTH.S41000>.
- Wen, R., Tao, W., Li, Y., Sieving, P.A., 2012. CNTF and retina. *Prog. Retin. Eye Res.* 31, 136–151. <https://doi.org/10.1016/j.preteyeres.2011.11.005>.

## CHAPTER

## 6.2

### Age-related macular degeneration

Age-related macular degeneration (ARMD) is an eye disease that is a leading cause of vision loss in older people in developed countries. The vision loss usually becomes noticeable in a person's sixties or seventies and tends to worsen over time. ARMD has an estimated prevalence of 1 in 2,000 people in the United States and other developed countries. (*Genetics Home Reference*, <https://ghr.nlm.nih.gov>).

ARMD is an acquired degeneration of the retinal pigment epithelium and subsequently the neurosensory retina and choroid resulting in loss of central vision. The etiology is not known but a combination of genetic predisposition and certain risk factors plays an important role. (*Ferri and Ferri, 2018*).

The pathogenesis of ARMD is not entirely clear. Complement activation, oxidative stress, all-trans-retinal, and lipofuscin toxicity play a role in the formation of yellow deposits of lipids and proteins (termed drusen) under the retina, retinal pigment epithelial (RPE) cell death, and inflammation activation:

**Pathway 1.** Complement activation in age-related macular degeneration (Fig. 4).

**Pathway 2.** Oxidative stress, all-trans-retinal and lipofuscin toxicity in age-related macular degeneration (Fig. 5).

Endoplasmic reticulum stress makes an impact on RPE cell death, inflammation, and neovascularization of the retina:

**Pathway 3.** Endoplasmic reticulum stress in age-related macular degeneration (Fig. 6).

Other mechanisms are still under investigation.

## Key cellular contributors and processes

Complement system

Protein or gene

The complement system is a group of small proteins that “complement” the ability of the antibody system to eliminate cellular pathogens. The liver produces the complement system proteins, which circulate in the blood as inactive precursors promoting inflammation and attack the pathogen’s plasma membrane.

Drusen formation

Process

Drusen are small yellow or white accumulations of extracellular material in the macula (a part of the retina) between the Bruch’s membrane and retinal pigment epithelium of the eye, which consist of proteins and lipids. The presence of a few small drusen is usual; however, the buildup of larger numerous drusen in the macula is a hallmark of age-related macular degeneration (ARMD).

Endoplasmic reticulum stress response

Process

The endoplasmic reticulum protein response (unfolded protein response, UPR) is a highly conserved adaptive process in eukaryotes triggered by a buildup of unfolded and/or misfolded proteins in the endoplasmic reticulum lumen. The UPR leads to restoration of normal cellular functioning or elimination of a severely damaged cell via apoptosis.

Lipofuscin aggregation

Process

Incomplete degradation of proteins inside lysosomes leads to the accumulation of the granules with insoluble autofluorescent pigment lipofuscin. Lipofuscin inhibits intracellular proteasomal system and lysosomal-autophagic systems.

Oxidative stress

Process

Oxidative stress occurs when the antioxidant defense system is unable to neutralize the harmful effects of ROS.

### Retinal pigment epithelial cells

#### Cell

Retinal pigment epithelial (RPE) cells are a single layer of specialized pigmented cells located between the retinal photoreceptor (PR) cells and the choroid. The RPE cells are essential for the maintenance of the retina—their functions include nourishment of the PR cells, absorption of excessive light, reisomerization and storage of the retinoid, and phagocytosis of shed PR membranes. The RPE cells are derived from the ectoderm and are considered part of the retina.

## Pathway 1

### Complement activation in age-related macular degeneration [\(Fig. 4\)](#)

#### Incoming signals

Experimental and clinical evidence strongly indicate the pathogenic role of immunological processes in ARMD occurrence, consisting of the production of related inflammatory molecules, recruitment of macrophages, complement activation, and microglial activation. All three of the complement pathways (classical, alternative, and lectin-mediated) are involved in the pathogenesis of ARMD.

#### Outcome effects

Complement proteins and other molecules take part in drusen formation. Drusen are small yellow or white accumulations of extracellular material, proteins and lipids, in the macula (a part of the retina) between the Bruch's membrane and the retinal pigment epithelium of the eye. The presence of a few small drusen is normal with advancing age. However, the buildup of larger and more numerous drusen in the macula is a hallmark of ARMD. Also, complement activation may induce retinal pigment epithelial (RPE) cell death, and neutrophil and macrophage activation.

#### Signaling

Complement pathway components implicated in ARMD pathogenesis include C3 and C5; the C5b-9 terminal complement complex; complement regulators or inhibitors, that is, complement factor H (CFH), vitronectin (VTN), and clusterin (CLU); complement receptor 1 (CR1); membrane cofactor protein (CD46); and decay accelerating factor (CD55). These components are systemically elevated in patients suffering from ARMD and are found in the retinal drusen. Numerous studies revealed significant correlations between ARMD and polymorphisms of genes encoding several molecules involved in complement pathways (e.g., CFH, C3, CFI, CFB, C2, and CFHR1/3). Mutations in the gene encoding the HtrA serine peptidase 1 (*HTRA1*) are one of the significant hereditary factors predisposing to ARMD. The *HTRA1* protein is a secreted enzyme that cleaves substrates involved in the complement pathway, including VTN and CLU, and thus it is implicated in the pathogenesis of ARMD. The following complement activators are also founding drusen: amyloid-beta, lipofuscin constituents, C-reactive protein (CRP), cholesterol, immunoglobulin G, and others (Anderson et al., 2010; Ardeljan and Chan, 2013; Charbel Issa et al., 2011; Parmeggiani et al., 2013; Weber et al., 2014).

## II. Human disease pathways

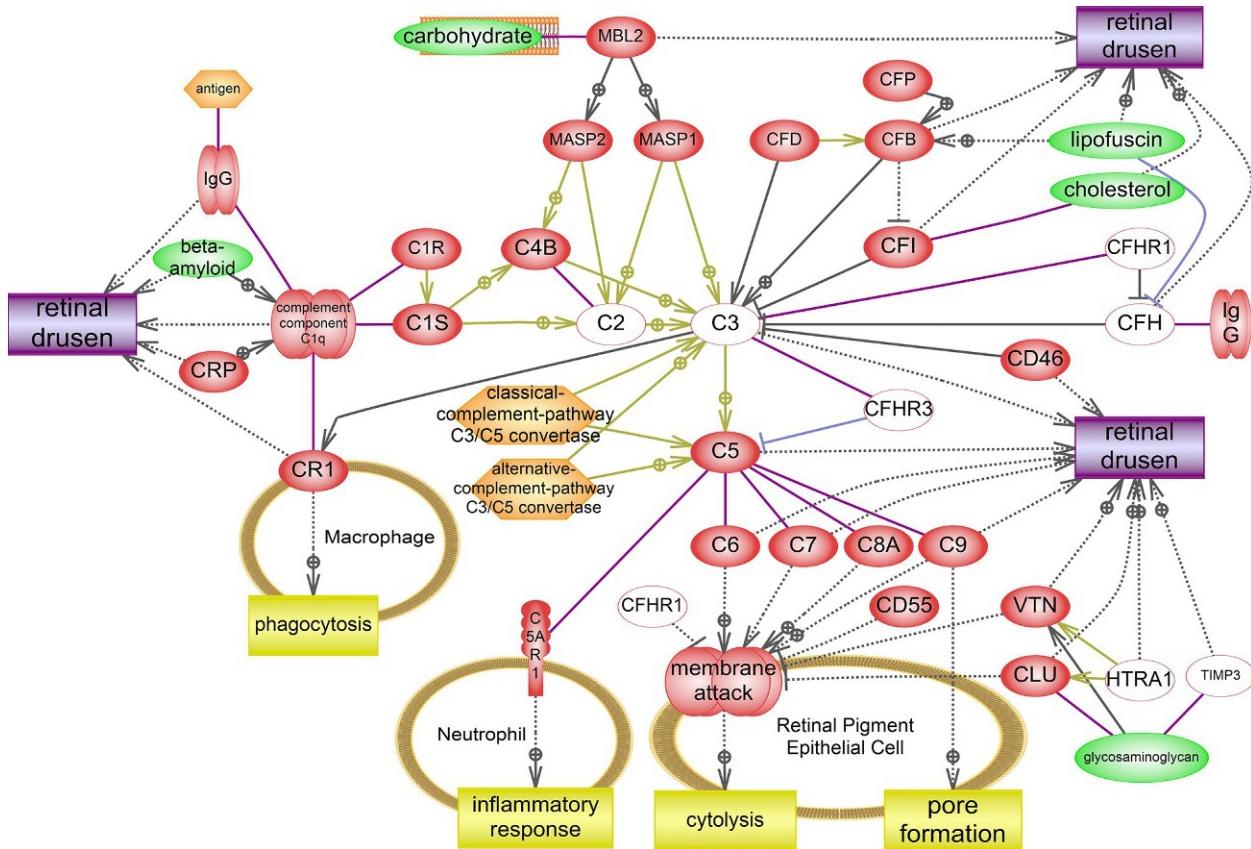


FIG. 4 Pathway 1: Complement activation in age-related macular degeneration.

## Pathway 2

### Oxidative stress, all-trans-retinal, and lipofuscin toxicity in age-related macular degeneration ([Fig. 5](#))

#### Incoming signals

Oxidative stress is regarded as one of the major factors in the development of ARMD. Cigarette smoking is also a well-known risk factor because it generates a lot of chemical components, some of which are strong oxidants. A high flux of retinoids through the retinoid cycle, which occurs during intense light exposure, can cause elevated levels of toxic retinoid intermediates, especially all-trans-retinal.

#### Outcome effects

Oxidative stress and the toxicity of all-trans-retinal and lipofuscin may lead to RPE cell apoptosis, retinal inflammation, autoimmune response, retinal drusen formation or choroidal neovascularization resulting in retina degradation, and vision loss. Lipofuscin granules are by-products of photoreceptor outer segment turnover and are seen within both healthy and pathological RPE cells. Lipofuscin in RPE cells accumulates throughout life and is readily detectable after age 40. Accumulation of lipofuscin results in a significant increase in retinal phototoxicity.

#### Signaling

All-trans-retinal was found to induce NADPH oxidase-mediated reactive oxygen species (ROS) generation. It has been shown that all-trans-retinal might act via the G protein-coupled 5-hydroxytryptamine receptor 2A (HTR2A) and cholinergic receptor muscarinic 3 (CHRM3) to induce NADPH oxidase.

While ROS are short lived, oxidatively damaged molecules termed oxidation-specific epitopes (OSEs) can be long-lived and provide a source of chronic stress that activates the immune system.

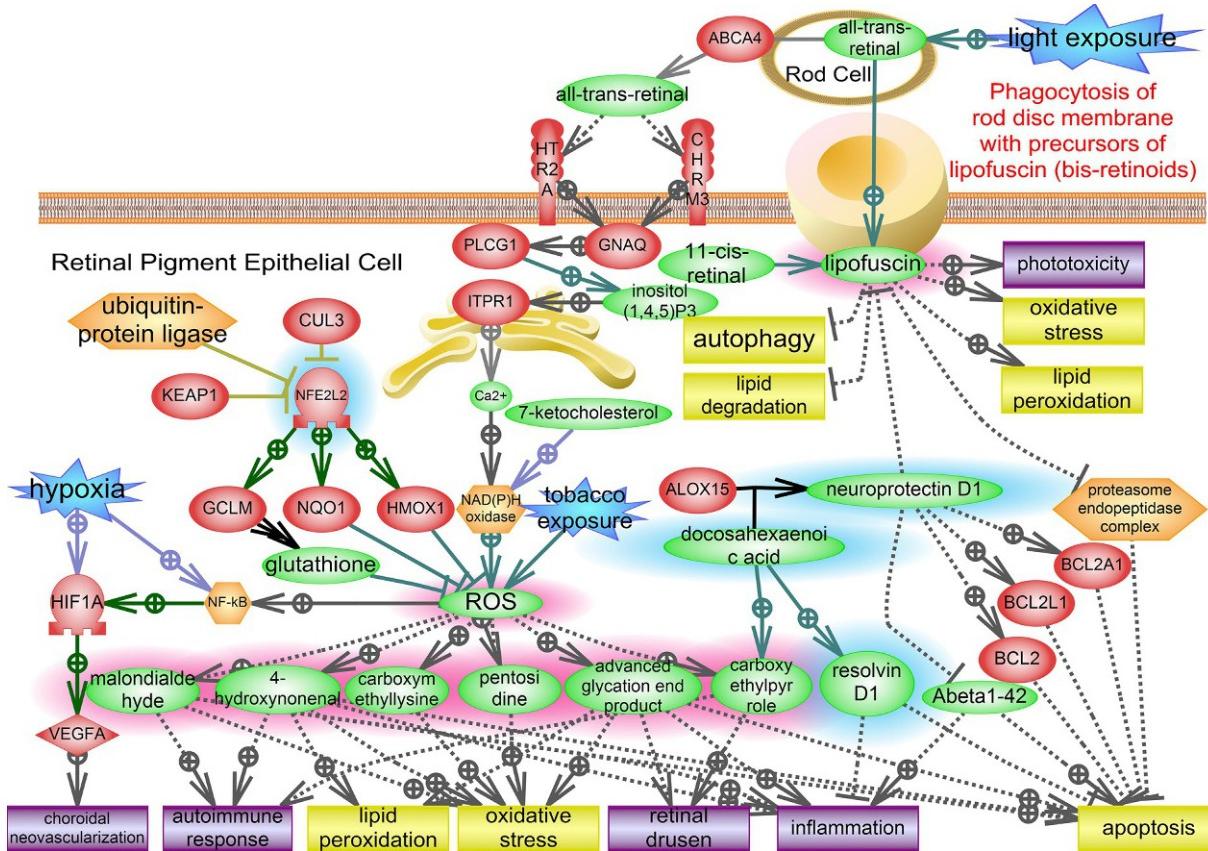
Oxidative damage, mediated by reactive oxygen species, generates harmful reactive aldehydic by-products including carboxyethylpyrrole (CEP), malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), and advanced glycation end (AGE) products such as carboxymethyllysine and pentosidine. Proteins modified with aldehydic (MDA and 4-HNE) or CEP adducts become more immunogenic. Therefore oxidation of lipids and proteins could induce the generation of new autoantigens to which the immune system has not been “tolerized.” CEP is a by-product generated from the oxidation of docosahexaenoic acid (DHA) in the retina.

DHA is typically a precursor of the neuroprotective factors neuroprotectin D1 and resolvin D1 that generally inhibit apoptosis and inflammation. Oxidative damage in the retina is capable of diverting DHA normally used in these protective functions toward the generation of pathogenic intermediates. In healthy cells, neuroprotectin D1 represses amyloid-beta-42-triggered activation of proinflammatory genes and upregulates the antiapoptotic genes encoding B-cell CLL/lymphoma 2 (*BCL2*), *BCL2* like 1 (*BCL2L1*), and *BCL2*-related protein A1 (*BCL2A1*). When the level of neuroprotectin D1 decreases, this does not happen.

Lipofuscin pigments are generated from random nonenzymatic reactions involving retinaldehyde in photoreceptors throughout life. They enter RPE via phagocytosis leading to the fusion of pigment-containing phagosomes with lysosomes. However, the random nature of lipofuscin pigment generation results in compounds that evade lysosomal digestion and thus accumulate within the RPE.

RPE cells have developed a robust antioxidant system driven by the transcription nuclear factor erythroid 2-like 2 (NFE2L2). It regulates antioxidant encoding genes coding proteins with antioxidant activity like heme oxygenase 1 (HMOX1), NAD(P)H quinone dehydrogenase 1 (NQO1), and glutamate-cysteine ligase modifier subunit (GCLM). Aging can reduce NFE2L2 activity. Impaired NFE2L2 signaling can lead to oxidative damage and in turn RPE cell apoptosis.

The transcription factor hypoxia-inducible factor 1 alpha subunit (HIF1A) was shown to be induced by ROS and hypoxia in AMD, and it controls expression of the vascular endothelial growth factor A gene (VEGFA) making an impact on choroidal neovascularization (Ardeljan et al., 2011; Ardeljan and Chan, 2013; Arjamaa et al., 2009; Camelo, 2014; Cano et al., 2010; Chen et al., 2012; Handa, 2012; Powell et al., 2005; Sachdeva et al., 2014; Salomon et al., 2011).



**FIG. 5** Pathway 2: Oxidative stress, all-trans-retinal and lipofuscin toxicity in age-related macular degeneration.

## Pathway 3

### Endoplasmic reticulum stress in age-related macular degeneration (Fig. 6)

#### Incoming signals

Oxidized low-density lipoproteins (LDL), homocysteine and benzopyrene, a component of cigarette smoke, lead to the endoplasmic reticulum (ER) stress and activation of the apoptosis of retinal pigment epithelial (RPE) cells.

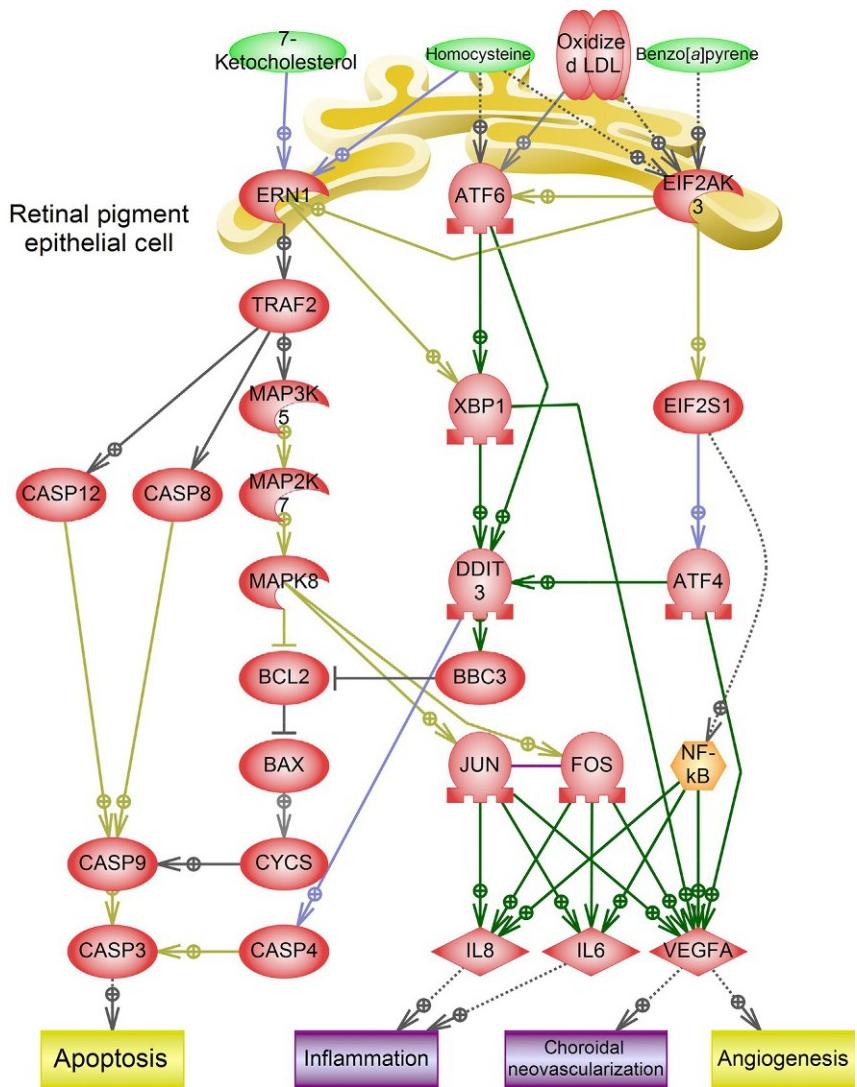
#### Outcome effects

Under ER and oxidative stress, retinal pigment epithelial (RPE) cells produce a high level of vascular epithelial growth factor A (VEGFA) and chemokines. VEGFA promotes neovascularization; interleukin 6 (IL6) and C-X-C motif chemokine ligand 8 (CXCL8) impact retinal inflammation. Apoptosis of RPE cells following oxidative stress leads to retina degradation and vision loss (Binet and Sapieha, 2015; Salminen et al., 2010; Sano and Reed, 2013; Zhang et al., 2015).

#### Signaling

7-Ketocholesterol and homocysteine induce ERN1 signaling. ERN1 signaling, in turn, leads to the activation of caspases resulting in apoptosis.

External toxins activate EIF2AK3/ATF4 signaling during ER stress in RPE cells. Activated eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3) phosphorylates the eukaryotic translation initiation factor 2 subunit alpha (EIF2S1), which in turn contributes to the activation of nuclear factor kB (NF-kB), a potent transcription factor that regulates angiogenesis and inflammation in proliferative retinal diseases.



**FIG. 6** Pathway 3: Endoplasmic reticulum stress in age-related macular degeneration.

## References

- Disease number numbers # 603075, # 153800, # 610698, # 610149, # 611378, # 613778, # 611953, # 613784, # 615439, # 615489 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code H35.3. Diseases of the eye and adnexa (H00-H59). (ICD-10, <https://icdlist.com>). ICD-11: disease code 9B75.0.
- Anderson, D.H., Radeke, M.J., Gallo, N.B., Chapin, E.A., Johnson, P.T., Curletti, C.R., Hancox, L.S., Hu, J., Ebright, J.N., Malek, G., Hauser, M.A., Rickman, C.B., Bok, D., Hageman, G.S., Johnson, L.V., 2010. The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited. *Prog. Retin. Eye Res.* 29, 95–112. <https://doi.org/10.1016/j.preteyeres.2009.11.003>.
- Ardeljan, D., Chan, C.-C., 2013. Aging is not a disease: distinguishing age-related macular degeneration from aging. *Prog. Retin. Eye Res.* 37, 68–89. <https://doi.org/10.1016/j.preteyeres.2013.07.003>.
- Ardeljan, D., Tuo, J., Chan, C.-C., 2011. Carboxyethylpyrrole plasma biomarkers in age-related macular degeneration. *Drugs Future* 36, 712–718.
- Arjamaa, O., Nikinmaa, M., Salminen, A., Kaarniranta, K., 2009. Regulatory role of HIF-1alpha in the pathogenesis of age-related macular degeneration (AMD). *Ageing Res. Rev.* 8, 349–358. <https://doi.org/10.1016/j.arr.2009.06.002>.
- Binet, F., Sapieha, P., 2015. ER Stress and Angiogenesis. *Cell Metab.* 22, 560–575. <https://doi.org/10.1016/j.cmet.2015.07.010>.
- Camelo, S., 2014. Potential sources and roles of adaptive immunity in age-related macular degeneration: shall we rename AMD into autoimmune macular disease? *Autoimmune Dis.* 2014, 532487. <https://doi.org/10.1155/2014/532487>.
- Cano, M., Thimmalappula, R., Fujihara, M., Nagai, N., Sporn, M., Wang, A.L., Neufeld, A.H., Biswal, S., Handa, J.T., 2010. Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration. *Vis. Res.* 50, 652–664. <https://doi.org/10.1016/j.visres.2009.08.018>.
- Charbel Issa, P., Chong, N.V., Scholl, H.P.N., 2011. The significance of the complement system for the pathogenesis of age-related macular degeneration—current evidence and translation into clinical application. *Graefes Arch. Clin. Exp. Ophthalmol.* 249, 163–174. <https://doi.org/10.1007/s00417-010-1568-6>.
- Chen, Y., Okano, K., Maeda, T., Chauhan, V., Golczak, M., Maeda, A., Palczewski, K., 2012. Mechanism of all-trans-retinal toxicity with implications for Stargardt disease and age-related macular degeneration. *J. Biol. Chem.* 287, 5059–5069. <https://doi.org/10.1074/jbc.M111.315432>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Handa, J.T., 2012. How does the macula protect itself from oxidative stress? *Mol. Asp. Med.* 33, 418–435. <https://doi.org/10.1016/j.mam.2012.03.006>.
- Parmegiani, F., Sorrentino, F.S., Romano, M.R., Costagliola, C., Semeraro, F., Incorvaia, C., D'Angelo, S., Perri, P., De Nadai, K., Bonomo Roversi, E., Franceschelli, P., Sebastiani, A., Rubini, M., 2013. Mechanism of inflammation in age-related macular degeneration: an up-to-date on genetic landmarks. *Mediat. Inflamm.* 2013, 435607. <https://doi.org/10.1155/2013/435607>.
- Powell, S.R., Wang, P., Divald, A., Teichberg, S., Haridas, V., McCloskey, T.W., Davies, K.J.A., Katzeff, H., 2005. Aggregates of oxidized proteins (lipofuscin) induce apoptosis through proteasome inhibition and dysregulation of proapoptotic proteins. *Free Radic. Biol. Med.* 38, 1093–1101. <https://doi.org/10.1016/j.freeradbiomed.2005.01.003>.
- Sachdeva, M.M., Cano, M., Handa, J.T., 2014. Nrf2 signaling is impaired in the aging RPE given an oxidative insult. *Exp. Eye Res.* 119, 111–114. <https://doi.org/10.1016/j.exer.2013.10.024>.

- Salminen, A., Kauppinen, A., Hyttinen, J.M., Toropainen, E., Kaarniranta, K., 2010. Endoplasmic reticulum stress in age-related macular degeneration: trigger for neovascularization. *Mol. Med.* 16, 535–542. <https://doi.org/10.2119/molmed.2010.00070>.
- Salomon, R.G., Hong, L., Hollyfield, J.G., 2011. Discovery of carboxyethylpyrroles (CEPs): critical insights into AMD, autism, cancer, and wound healing from basic research on the chemistry of oxidized phospholipids. *Chem. Res. Toxicol.* 24, 1803–1816. <https://doi.org/10.1021/tx200206v>.
- Sano, R., Reed, J.C., 2013. ER stress-induced cell death mechanisms. *Biochim. Biophys. Acta* 1833, 3460–3470. <https://doi.org/10.1016/j.bbamcr.2013.06.028>.
- Weber, B.H.F., Charbel Issa, P., Pauly, D., Herrmann, P., Grassmann, F., Holz, F.G., 2014. The role of the complement system in age-related macular degeneration. *Dtsch. Arztebl. Int.* 111, 133–138. <https://doi.org/10.3238/arztebl.2014.0133>.
- Zhang, S.X., Ma, J.H., Bhatta, M., Fliesler, S.J., Wang, J.J., 2015. The unfolded protein response in retinal vascular diseases: implications and therapeutic potential beyond protein folding. *Prog. Retin. Eye Res.* 45, 111–131. <https://doi.org/10.1016/j.preteyeres.2014.12.001>.

## CHAPTER

## 6.3

## Cataract

Cataract is the formation of a cloudy or opaque area in the normally clear lens of the eye that affects visual quality. A cataract develops due to the gradual accumulation of molecular modifications in crystallins, the major proteins of the lens, which changes crystallin function and leads to the formation of opacities in the lens.

Cataracts are the clouding and opacification of the normally clear crystalline lens of the eye. The opacity may occur in the cortex, the nucleus of the lens, or the posterior subcapsular region, but it is usually in a combination of areas. (*Ferri and Ferri, 2018*).

There exist several types of cataracts: A subcapsular cataract occurs at the back of the lens and may be triggered by diabetes or steroid medications. A nuclear cataract develops in the central zone (nucleus) of the lens and is related to aging. Age-related cataract (ARC) is the leading cause of blindness in older individuals. A cortical cataract develops in the lens cortex and forms white, wedge-like opacities.

A hereditary predisposition is the primary risk factor for cataract development. There are forms of congenital cataract that may occur at birth or develop at any age. Mutations in the crystallin alpha A (CRYAA), the gamma D-crystallin gene (CRYGD), and some other genes have been linked to cataracts (*Alapure et al., 2012; Galichanin et al., 2012; Reddy et al., 2004; Rhodes and Sanderson, 2009; Vinson, 2006*).

Since cataract is a heterogeneous disease, several different mechanisms have an impact on the development of the clinical features of the disease. Calcium ( $\text{Ca}^{2+}$ )-mediated toxicity in the lens cells is one of the major pathological mechanisms leading to different forms of cataract. Further, reactive oxygen species (ROS)-mediated and glucose-mediated toxicity are considered underlying factors in the formation of age-related cataract:

**Pathway 1. Age-related cataract (Fig. 7).**

Glucose-mediated toxicity is also considered to be one of the causes of diabetes-induced cataract:

**Pathway 2. Diabetes-induced cataract (Fig. 8).**

In most cases, the congenital cataract is caused by mutations in genes encoding the crystallins and gap junction proteins:

**Pathway 3. Congenital cataract (Fig. 9).**

## Key cellular contributors and processes

Intracellular sorbitol accumulation

Process

In diabetes, intracellular accumulation of the sugar alcohol sorbitol leads to changes in crystallin structure and, thus, accelerates cataract development.

Lens epithelial cells

Cell

The lens epithelium located on the anterior surface of the lens is composed of cuboidal-shaped epithelial cells, which regulate lens homeostasis.

Reactive oxygen species

Process

Reactive oxygen species (ROS) are chemically reactive oxygen-containing molecules commonly produced during normal metabolic processes that involve oxygen. ROS can damage all essential cellular components including lipids, proteins, and DNA.

## Pathway 1

### Age-related cataract (Fig. 7)

#### Incoming signals

Age-related cataract (ARC) is the leading cause of blindness in older individuals. In general, the pathology is described as the grouping of protein molecules, known as crystallins, to form large aggregates and the subsequent separation of the molecules due to water entry.

To date, the widely accepted theory for the origin of ARC is based on free radicals. Free radicals, like reactive oxygen species (ROS), are formed as a result of external and internal exposures. Common external factors include UV light exposure, ionizing radiation, and smoking, while the internal factors include a decrease in intracellular antioxidant defenses such as glutathione, superoxide dismutase 1 (SOD1), and ascorbate.

#### Outcome effects

The main molecular hypothesis describing triggers of age-related cataract includes ROS-induced membrane lipid peroxidation and direct impairment of structural lens proteins, that is, the crystallins. Lipid peroxidation disrupts the membranes of lens cells, changes the ion transport balance, and elevates intracellular calcium ( $\text{Ca}^{2+}$ ) and sodium ( $\text{Na}$ ) ions resulting in a  $\text{H}_2\text{O}$  influx into the cell, thereby provoking the separation of molecules and changes in light scattering.

The accumulation of the characteristic yellow-brown pigment along with disruption of the normal architecture of the lens fibers affects the quality of transmission of light through the lens causing visual problems.

#### Signaling

ROS are accumulated in lens cells due to a number of factors and attack the membrane lipids causing their peroxidation.

Several mechanisms are responsible for the accumulation of various ROS including oxygen ( $\text{O}_2$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and hydroxyl radicals.

Probably in older individuals, the levels of  $\text{Fe}_3$  and  $\text{Cu}_2$  are elevated. Thus the excessive amounts of  $\text{Fe}_3$  and  $\text{Cu}_2$  interact with ascorbate resulting in dehydroascorbate,  $\text{H}_2\text{O}_2$ ,  $\text{Fe}_2$ , and Cu formation. Subsequently,  $\text{H}_2\text{O}_2$ ,  $\text{Fe}_2$ , and Cu produce hydroxyl radicals.

In addition, smoking, another risk factor for cataracts, also may participate in ROS formation. Cd<sub>2+</sub>, present in tobacco smoke, enters the lens cells and replaces  $\text{Zn}^{2+}$  in the active center of the SOD1 protein. As a result,

SOD1 fails to detoxify O<sub>2</sub>. Further, growing evidence points to a decreased concentration of glutathione, a molecule involved in ROS detoxification, in most types of cataracts.

There are numerous consequences of ROS accumulation.

ROS attack membrane lipids promoting their peroxidation. Lipids peroxidation results in the dysfunction of Ca<sup>2+</sup>-ATPases and leads to the release of calcium from lipids.

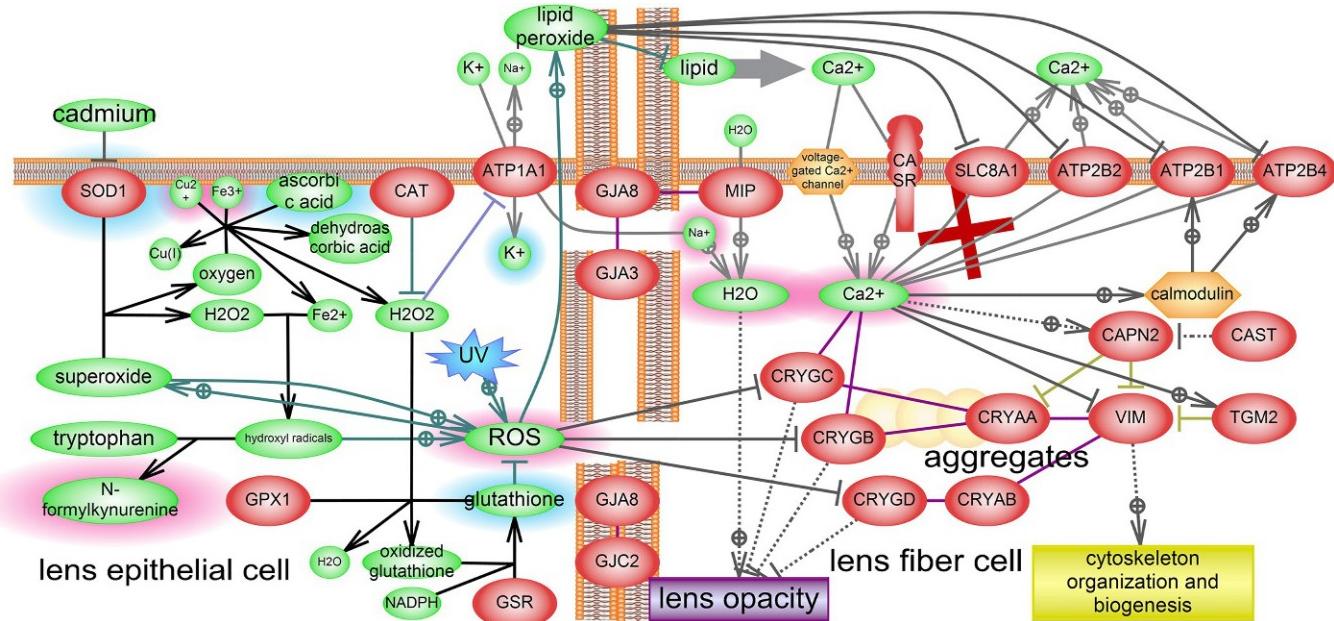
Normally, a high Ca<sup>2+</sup> concentration is observed in the human aqueous humor. Therefore calcium ions constantly enter the cell in an inwardly directed gradient across the plasma membrane of the lens cells. CASR and voltage-gated Ca<sup>2+</sup> channels promote free Ca<sup>2+</sup> entrance into the cell. There are several mechanisms that help maintain a normally low Ca<sup>2+</sup> concentration in lens cells. The Na/Ca<sup>2+</sup> exchanger SLC8A1 and Ca<sup>2+</sup>-ATPases, including ATP2B1-4 in healthy lenses, are activated by Ca<sup>2+</sup>-dependent calmodulin, thereby promoting a Ca<sup>2+</sup> efflux from the cell. Also, lipids bind ions on the outer membrane and decrease their concentration in the cell's vicinity.

Increased intracellular Ca<sup>2+</sup> binds crystallins leading to their aggregation. ROS also attack and damage crystallins directly.

High levels of intracellular calcium, in addition, activates calpains, preferentially calpain 2 (CAPN2), which cleave both vimentin (VIM) and crystallin alpha A (CRYAA). This leads to cytoskeleton disorganization in lens cell and a block of the activity of chaperones causing light scattering and lens opacities.

Further, oxygen radicals induce the degradation of intrinsic lens L-tryptophan to N-formylkynurenine, which increases the yellow to brown coloration of the lens nucleus (Bron et al., 2000; Gupta et al., 2004, 2014; Michael and Bron, 2011; Rhodes and Sanderson, 2009; Sanderson et al., 2000).

## II. Human disease pathways



**FIG. 7** Pathway 1: Age-related cataract.

## Pathway 2

### Diabetes-induced cataract (Fig. 8)

#### Incoming signals

Cataract is a common complication in diabetes. High levels of glucose in patients with diabetes lead to cataract formation by an accumulation of sorbitol and fructose, which are both capable of modifying the structural proteins of the lenses.

#### Outcome effects

High glucose levels cause the accumulation of sorbitol and advanced glycation end products. They cause an increase of water influx, thereby provoking the formation of lens opacities. Glucose and osmolar stress decrease antioxidant defenses in the lens and therefore promote ROS damage of cellular proteins and an induction of  $\text{Ca}^{2+}$  toxicity.

#### Signaling

Lens cells take up glucose in an insulin-independent process. Glucose enters the lens epithelium through the solute carrier family 2 member 1 (SLC2A1).

The glucose-induced elevation of diacylglycerol concentration leads to protein kinase C gamma (PRKCG) activation, which in turn phosphorylates gap junction protein alpha 3 (GJA3), thereby disrupting lens fiber gap junctions. This impairs water circulation, which then leads to its accumulation, thereby provoking lens opacities.

In diabetes, excess glucose enters the sorbitol pathway. In the first step, aldo-keto reductase family 1 member B (AKR1B1) catalyzes the NADPH-dependent reduction of glucose to its sugar alcohol, sorbitol. Then, sorbitol dehydrogenase (SORD) catalyzes the NAD-dependent oxidation of sorbitol to form fructose. Sorbitol is hydrophilic and does not diffuse through the cell membrane; therefore it accumulates intracellularly resulting in osmotic stress within cells of the lens.

The fructose produced by the polyol pathway can be phosphorylated to fructose-3-P, which is broken down into 3-deoxyglucosone. Both compounds are powerful glycosylating agents involved in the formation of advanced glycation end products. When those end products accumulate, they can modify structural proteins, such as the crystallins, in the lens, and provoke the formation of lens opacities.

Glucose pathways may decrease cofactor availability for glutathione reductase (GSR), an enzyme that is critical for the maintenance of the intracellular pool of reduced glutathione, an important antioxidant (Obrosova et al., 2010; Pollreisz and Schmidt-Erfurth, 2010).

## II. Human disease pathways

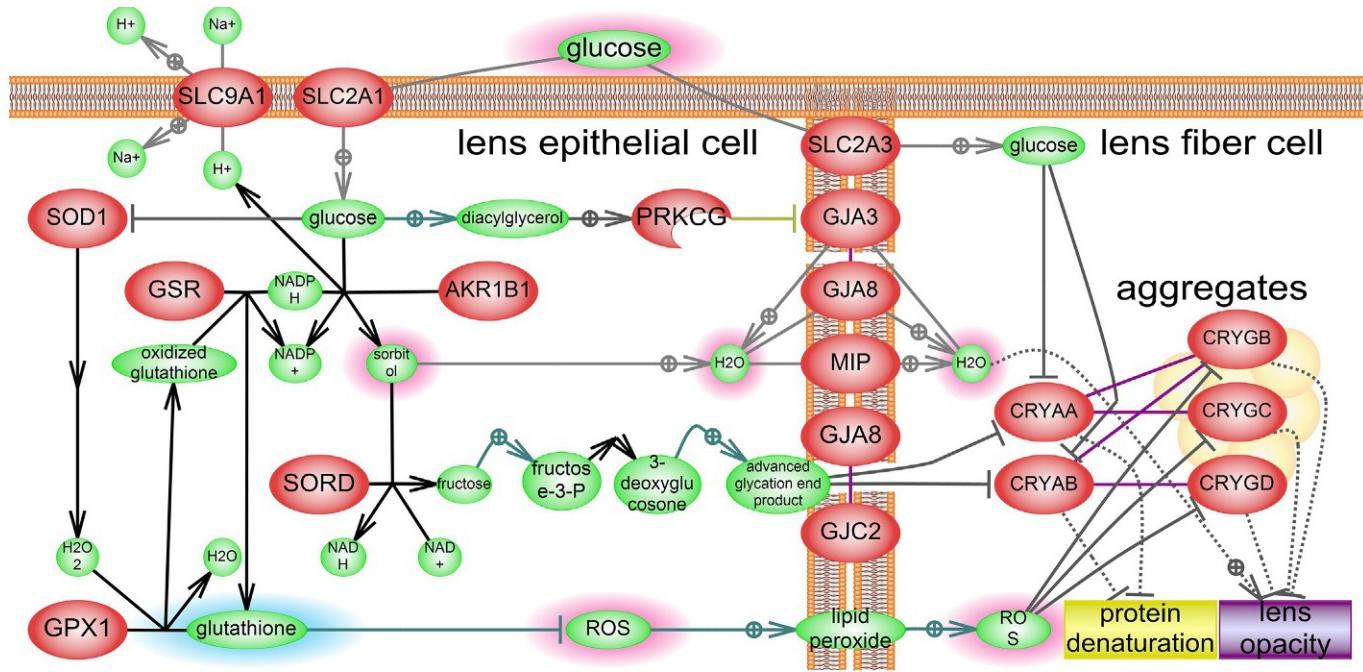


FIG. 8 Pathway 2: Diabetes-induced cataract.

## Pathway 3

### Congenital cataract (Fig. 9)

#### Incoming signals

Congenital cataract is a common cause of visual disability in children. Inherited isolated (nonsyndromic) cataract represents one-third of all observed cases. At least 22 genes associated with isolated inherited cataract have been identified including the crystallin genes, such as *CRYAA*, *CRYAB*, *CRYBB1*, *CRYBB2*, *CRYGC*, *CRYGD*, and *CRYGS*; four genes encoding membrane proteins including *GJA3*, *GJA8*, *MIP*, and *LIM2*; genes encoding transcription factors such as *MAF* and *HSF4*; and genes encoding cytoskeletal proteins including *BFSP1* and *BFSP2*.

Crystallins are critical proteins in the human eye lens, and they are responsible for the transparency and refractive index of the lens. Mammalian lens crystallins include alpha, beta, and gamma protein families.

#### Outcome effects

Mutations in these genes lead to a reduced accumulation of glutathione and ROS, which together participate in the formation of different types of cataract. Mutations in the *MIP* gene lead to a separation of the lens structural molecules due to water accumulation within the lens cells. Also, impaired crystallin gene expression is observed when *MAF* is mutated.

#### Signaling

The molecular mechanisms of congenital cataract development involve either protein misfolding, as seen in the case of mutations in the crystallins *CRYBB1*, *CRYBB2*, *CRYGC*, *CRYGD*, and *CRYGS*, or aggregation caused by mutations in the *CRYAA* and *CRYAB* genes.

The beta-crystallins B1/2 (*CRYBB1/2*) form aggregates of different sizes and can self-associate to form dimers or heterodimers with other beta-crystallins.

The crystallin alpha A (*CRYAA*) and crystallin alpha B (*CRYAB*) act as specific molecular chaperones, which maintain proteins in large soluble aggregates. *CRYAA* and *CRYAB* are differentially expressed. Alpha A is expressed in the lens, and alpha B is expressed widely in many tissues and organs. Defects in the *CRYAA* gene cause autosomal dominant congenital cataract.

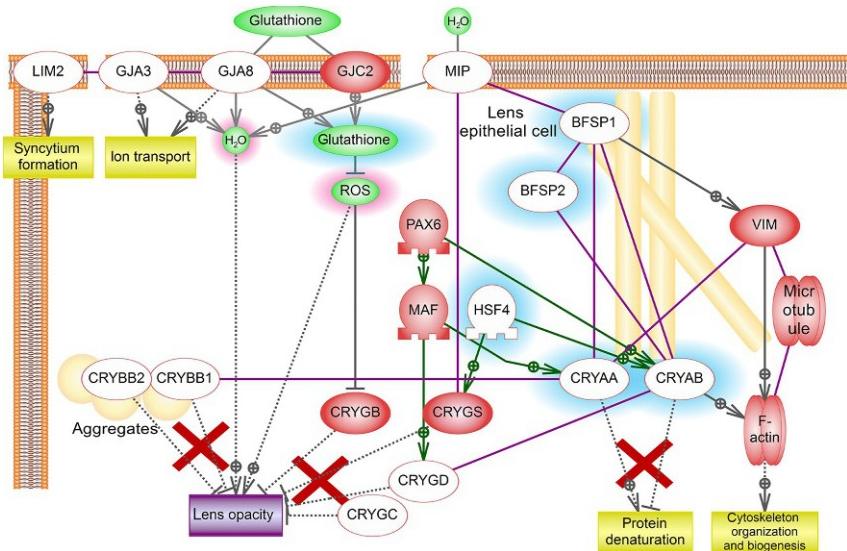
The gamma-crystallins, crystallin gamma C (*CRYGC*), crystallin gamma D (*CRYGD*), and crystallin gamma S (*CRYGS*) are highly symmetrical, monomeric proteins differentially regulated after early development. Mutations in the *CRYGC*, *CRYGD*, and *CRYGS* genes have been shown to cause multiple types of cataract.

The mechanism of congenital cataract pathogenesis may be based on the disruption of the processes of molecular transport between lens cells due to mutations in the genes encoding aquaporin (MIP) and the gap junction proteins gap junction protein alpha 3 and 8 (GJA3 and GJA8). These proteins, when carrying loss-of-function mutations, cause reduced glutathione levels, thus triggering the ROS accumulation. Major intrinsic protein (MIP) of lens fiber is a water-transporting aquaporin channel. Mutations in the MIP gene lead to separation of the lens structural proteins due to water accumulation within lens cells.

Beaded filaments including BFSP1 and BFSP2 are lens cell-specific intermediate filaments, which help maintain lens transparency. Mutations in these genes lead to cytoskeleton disorganization and increased light scattering.

Lastly, impaired crystallin expression is observed when the transcription factor genes MAF and HSF4 are mutated. MAF (MAF bZIP transcription factor) regulates several cellular processes including embryonic lens fiber cell development. Heat shock transcription factor 4 (HSF4) activates heat shock response genes under conditions of elevated heat or other stresses.

Finely, lens intrinsic membrane protein 2 (LIM2) probably controls the organization of eye cell junctions. LIM2 acts as a receptor for calmodulin and can be involved in lens development (Bhat, 2003; Carter et al., 2000; Dudakova et al., 2017; Enoki et al., 2010; Graw, 2004; Messina-Baas and Cuevas-Covarrubias, 2017; Perveen et al., 2007; Ramachandran et al., 2007; Santana et al., 2009; Shi et al., 2009).



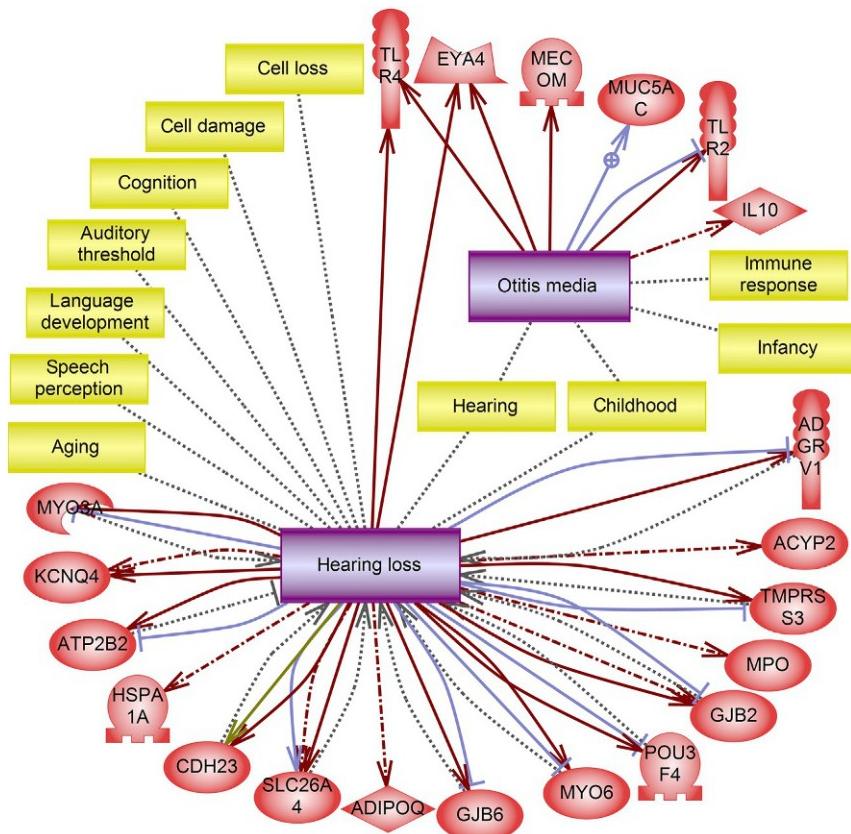
**FIG. 9** Pathway 3: Congenital cataract.

## References

- Disease number # 116200, # 115700, # 604219 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code H26.9/H26.2. Diseases of the eye and adnexa (H00-H59). (ICD-10, <https://icdlist.com>). ICD-11: disease code 9B10.0/LA12.1.
- Alapure, B.V., Praveen, M.R., Gajjar, D.U., Vasavada, A.R., Parmar, T.J., Arora, A.I., 2012. Matrix metalloproteinase-2 and -9 activities in the human lens epithelial cells and serum of steroid induced posterior subcapsular cataracts. *Mol. Vis.* 18, 64–73.
- Bhat, S.P., 2003. Crystallins, genes and cataract. *Prog. Drug Res.* 60, 205–262.
- Bron, A.J., Vrensen, G.F.J.M., Koretz, J., Maraini, G., Harding, J.J., 2000. The ageing lens. *Ophthalmologica* 214, 86–104. <https://doi.org/10.1159/000027475>.
- Carter, J.M., McLean, W.H.I., West, S., Quinlan, R.A., 2000. Mapping of the human CP49 gene and identification of an intragenic polymorphic marker to allow genetic linkage analysis in autosomal dominant congenital cataract. *Biochem. Biophys. Res. Commun.* 270, 432–436. <https://doi.org/10.1006/bbrc.2000.2442>.
- Dudakova, L., Stranecky, V., Ulmanova, O., Hlavova, E., Trková, M., Vincent, A.L., Liskova, P., 2017. Correction to: segregation of a novel p.(Ser270Tyr) MAF mutation and p.(Tyr56\*) CRYGD variant in a family with dominantly inherited congenital cataracts. *Mol. Biol. Rep.* 44, 441. <https://doi.org/10.1007/s11033-017-4130-3>.
- Enoki, Y., Mukoda, Y., Furutani, C., Sakurai, H., 2010. DNA-binding and transcriptional activities of human HSF4 containing mutations that associate with congenital and age-related cataracts. *Biochim. Biophys. Acta (BBA) Mol. Basis Dis.* 1802, 749–753. <https://doi.org/10.1016/j.bbadiis.2010.06.001>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Galichanin, K., Talebizadeh, N., Söderberg, P., 2012. Characterization of molecular mechanisms of in vivo UVR induced cataract. *J. Vis. Exp.*, e4016. <https://doi.org/10.3791/4016>.
- Graw, J., 2004. Congenital hereditary cataracts. *Int. J. Dev. Biol.* 48, 1031–1044. <https://doi.org/10.1387/ijdb.041854jg>.
- Gupta, P.D., Johar, K., Vasavada, A., 2004. Causative and preventive action of calcium in cataracto-genesis. *Acta Pharmacol. Sin.* 25, 1250–1256.
- Gupta, V., Rajagopala, M., Ravishankar, B., 2014. Etiopathogenesis of cataract: an appraisal. *Indian J. Ophthalmol.* 62, 103. <https://doi.org/10.4103/0301-4738.121141>.
- Messina-Baas, O., Cuevas-Covarrubias, S.A., 2017. Inherited congenital cataract: a guide to suspect the genetic etiology in the cataract genesis. *Mol. Syndromol.* 8, 58–78. <https://doi.org/10.1159/000455752>.
- Michael, R., Bron, A.J., 2011. The ageing lens and cataract: a model of normal and pathological ageing. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 366, 1278–1292. <https://doi.org/10.1098/rstb.2010.0300>.
- Obrosova, I.G., Chung, S.S.M., Kador, P.F., 2010. Diabetic cataracts: mechanisms and management. *Diabetes Metab. Res. Rev.* 26, 172–180. <https://doi.org/10.1002/dmrr.1075>.
- Perveen, R., Favor, J., Jamieson, R.V., Ray, D.W., Black, G.C.M., 2007. A heterozygous c-Maf transactivation domain mutation causes congenital cataract and enhances target gene activation. *Hum. Mol. Genet.* 16, 1030–1038. <https://doi.org/10.1093/hmg/ddm048>.
- Pollreisz, A., Schmidt-Erfurth, U., 2010. Diabetic cataract-pathogenesis, epidemiology and treatment. *J. Ophthalmol.* 2010, 608751. <https://doi.org/10.1155/2010/608751>.
- Ramachandran, R.D., Perumalsamy, V., Heitmancik, J.F., 2007. Autosomal recessive juvenile onset cataract associated with mutation in BFSP1. *Hum. Genet.* 121, 475–482. <https://doi.org/10.1007/s00439-006-0319-6>.
- Reddy, M.A., Francis, P.J., Berry, V., Bhattacharya, S.S., Moore, A.T., 2004. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv. Ophthalmol.* 49, 300–315. <https://doi.org/10.1016/j.survophthal.2004.02.013>.

- Rhodes, J.D., Sanderson, J., 2009. The mechanisms of calcium homeostasis and signalling in the lens. *Exp. Eye Res.* 88, 226–234. <https://doi.org/10.1016/j.exer.2008.10.025>.
- Sanderson, J., Marcantonio, J.M., Duncan, G., 2000. A human lens model of cortical cataract:  $\text{Ca}^{2+}$ -induced protein loss, vimentin cleavage and opacification. *Invest. Ophthalmol. Vis. Sci.* 41, 2255–2261.
- Santana, A., Waiswol, M., Arcieri, E.S., Cabral de Vasconcellos, J.P., Barbosa de Melo, M., 2009. Mutation analysis of CRYAA, CRYGC, and CRYGD associated with autosomal dominant congenital cataract in Brazilian families. *Mol. Vis.* 15, 793–800.
- Shi, X., Cui, B., Wang, Z., Weng, L., Xu, Z., Ma, J., Xu, G., Kong, X., Hu, L., 2009. Removal of Hsf4 leads to cataract development in mice through down-regulation of  $\gamma$ S-crystallin and Bfsp expression. *BMC Mol. Biol.* 10, 10. <https://doi.org/10.1186/1471-2199-10-10>.
- Vinson, J.A., 2006. Oxidative stress in cataracts. *Pathophysiology* 13, 151–162. <https://doi.org/10.1016/j.pathophys.2006.05.006>.

# Diseases of the ear



## O U T L I N E

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Diseases of the ear are as diverse as diseases of the eye and can affect both hearing and the sense of balance. Ear diseases can be classified by the part of the ear affected, namely, the outer, middle, or the inner ear.

In children with normal hearing, inflammatory disorders caused by infections of the middle ear (otitis media) are the most common ear illnesses. Inflammation of the middle ear often causes acute pain, and untreated otitis media may lead to severe complications such as perforation of the eardrum or even bacterial meningitis.

Many of older adults experience some level of hearing loss, either a partial loss or the total inability to hear (deafness).

Several factors can lead to either a partial loss or the total inability to hear (deafness) including exposure to noise, a hereditary predisposition, chronic infections, traumas, medications, and aging. In nonsyndromic hearing loss, there are any associations of loss of hearing with additional manifestations. In contrast, syndromic hearing loss occurs with signs and symptoms affecting other parts of the body. Hearing loss is often inherited with approximately 75%–80% of observed cases inherited in a recessive manner and 20%–25% with a dominant mode of inheritance.

## CHAPTER

## 7.1

## Hearing loss

Hearing loss is a complex condition. The nonsyndromic hearing loss is a partial or total loss of hearing not associated with other signs and symptoms. In contrast, syndromic hearing loss occurs with signs and symptoms affecting other parts of the body (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Nonsyndromic hearing loss is classified in several different ways, for example, by the pattern of inheritance (autosomal dominant, autosomal recessive, X-linked, or mitochondrial). The causes of nonsyndromic hearing loss are complex with mutations in more than 90 genes associated with nonsyndromic hearing loss to date. Many of these genes handle the development and function of the inner ear.

Age-related hearing loss (ARHL, also known as presbycusis) is a decrease in hearing ability that happens with age. ARHL develops from a combination of genetic, environmental, and lifestyle factors. Age-related hearing loss is most commonly related to dysfunctions in the inner ear, where sound waves turn into nervous impulses (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Mutations in genes encoding structural proteins specific for cochlear hair cell may cause hearing loss:

**Pathway 1.** *Dysfunction of cochlear hair cell stereocilia proteins in hearing loss (Fig. 1).*

**Pathway 2.** *Dysfunction of cochlear hair cell synapse proteins in hearing loss (Fig. 2).*

Impairment of mechanoelectrical transduction and potassium ( $K^+$ ) cycling in the inner ear is the main reason for congenital hearing loss:

**Pathway 3.** *Deficiency of potassium cycling in hearing loss (Fig. 3).*

## Key cellular contributors and processes

### Cochlear hair cell

#### Cell

Cochlear hair cells are the sensory cells of the auditory system. These cells possess stereocilia connected to the tectorial membrane. During auditory stimulation, sound waves in the cochlea cause deflection of the hair cell stereocilia, which creates an electrical signal in the hair cell.

### Cochlear

#### Anatomic structure

Cochlea is a snail-shaped canal in the osseous labyrinth of the inner ear, which contains the sensory organ of hearing—the organ of Corti.

### Inner ear

#### Anatomic structure

The inner ear is the innermost portion of the ear that contains organs responsible for hearing and the sense of balance. Located in the temporal bone, the inner ear has three essential parts: cochlea, vestibule, and semi-circular canals.

### Mechanoelectrical transducer channel

#### Anatomic structure

The mechanoelectrical transducer (MET) channels are ion channels on the tips of stereocilia. Deflection of stereocilia provokes mechanical opening of these channels and the entrance of cations that generates action potential.

### Organ of Corti

#### Anatomic structure

The organ of Corti is the auditory organ situated in the cochlea of the inner ear. The sensory hair cells that make up the organ of Corti are responsible for the transduction of the auditory impulse into neural signals.

### Ribbon synapses

#### Cell

A ribbon synapse is a neuronal synapse structurally different from other synapses by the presence of an electron-dense structure called synaptic ribbon, which helps to keep synaptic vesicles near the active zone. Ribbon synapses are found in various sensory receptor cells, for example, auditory hair cells of the cochlea, and characterized by increased performance.

## Stereocilia

### Anatomic structure

Stereocilia are thin projections on the cochlear hair cells that respond to fluid motion and are involved in mechanosensing. Despite a similar name, stereocilia are different from cilia (microtubule cytoskeleton-based structures) and contain actin cytoskeleton, similarly to microvilli.

## Tectorial membrane

### Anatomic structure

The tectorial membrane is a band of extracellular matrix in the cochlea located above the inner and outer hair cells of the organ of Corti. The tectorial membrane is connected to stereocilia of the outer hair cells and participates in mechanotransduction. During auditory stimulation the tectorial membrane directly stimulates the outer hair cells and creates liquid movements that stimulate the inner hair cells.

## Pathway 1

### Dysfunction of cochlear hair cell stereocilia proteins in hearing loss (Fig. 1)

#### Incoming signals

The transduction of sound waves within the ear involves movement of parts of the cochlea in the inner ear including the tectorial membrane and the fluid within the labyrinth termed endolymph. Endolymph, found inside the cochlear duct (i.e., the scala media), is very rich in potassium (150 mM) and very poor in sodium (1 mM). These concentrations are unique among physiological fluids. Hearing depends on the high K<sup>+</sup> concentration in endolymph. Fluid motion and tectorial membrane vibrations bend protrusions of hair cell membranes (stereocilia). Stereocilia movements and K<sup>+</sup> and Ca<sup>2+</sup> influx transform mechanical impulses (i.e., sound waves) into electrical impulses in the form of action potentials. Loss-of-function mutations in different genes that encode critical proteins in stereocilia of the cochlear hair cell impair mechanoelectrical transduction and therefore cause hearing loss. Congenital hearing loss is most often associated with dysfunction of actin-myosin complex organization within the ear. The pathway reconstructed here reviews all known mutations together although usually one mutated gene underlies inborn hearing loss.

#### Outcome effects

Bending of higher stereocilia under the influence of a sound wave causes mechanical opening of the mechanoelectrical transducer (MET) channels on the membranes of lower stereocilia by tensioning the tip of each lower stereocilium with the side wall of its associated higher one. K<sup>+</sup> and Ca<sup>2+</sup> enter the stereocilium through MET channels and lead to the transformation of the mechanical impulse or sound wave into an electrical impulse or action potential. Dysfunctions in stereocilia proteins lead to the impairment of their movements, the inability of mechanoelectrical transducer channels to open, and the subsequent failure to transform a sound wave into an electric impulse.

#### Signaling

Stereocilia movement is an actin-/myosin-dependent process. The loss of function of a number of myosins (such as MYO3A, MYO6, MYO7A, MYO15A, MYO1A, MYO1C, MYO1F, MYH9, and MYH14) has been shown to be associated with both dominant and recessive forms of hearing

loss. *MYO7A* mutations, for example, may cause a rare disorder known as Usher syndrome type IB.

Dysfunction of several proteins controlling actin filaments in the cytoskeleton may be the reason for some subtypes of nonsyndromic hearing loss. Homer scaffolding protein 2 (HOMER2) regulates actin dynamics in stereocilia through its interaction with the cell division cycle 42 (CDC42) protein. Diaphanous-related formin 1 (DIAPH1) controls the actin polymerization. Taperin (TPRN) modulates actin dynamics through direct or indirect contact with the ends of actin filaments. Chloride intracellular channel 5 (CLIC5) stabilizes membrane-actin filament linkages at the base of hair cell stereocilia as part of a molecular complex with radixin (RDX), TPRN, and myosin VI (MYO6). The protein tyrosine phosphatase receptor type Q (PTPRQ) hydrolyzes 4,5-phosphatidylinositol bisphosphate (PIP<sub>2</sub>), a key regulator of actin remodeling. TRIO and the F-actin binding protein (TRIOBP) stabilizes F-actin structures. Finally, when the core of the actin filament known as actin gamma 1 (ACTG1) is altered, the autosomal dominant form of hearing loss develops.

Dysfunction in cell-cell adhesion protein complexes also may cause instances of autosomal recessive deafness. Otoferlin (OTOF) and otoancorin (OTOA) are important proteins for the attachment of acellular gels to the underlying nonsensory cells in the inner ear. The MARVEL domain containing 2 (MARVELD2), tight junction protein 2 (TJP2), and claudin 14 (CLDN14) together provide regular tight junction assemblies. A carcinoembryonic antigen-related cell adhesion molecule 16 (CEACAM16) on the tips of the higher stereocilia and the tectorial membrane (TM) protein alpha-tectorin (TECTA) are essential for maintaining the integrity of the tectorial membrane and for the association of stereocilia with the TM. Dysfunctional CEACAM16 or TECTA cause autosomal dominant nonsyndromic deafness and a recessive form of sensorineural prelingual nonsyndromic deafness (TECTA).

Solute carrier family 26 (anion exchanger) member 5 (SLC26A5, also known as prestin) shuttles chloride ions across the cell membrane and undergoes a conformational change in response to changes in intracellular Cl<sup>-</sup> levels leading to electromotility of outer hair cells.

The cadherin-related 23 (CDH23) protein and protocadherin-related 15 (PCDH15) play a major role in forming a tip link between the top of a shorter stereocilium and the side of the nearby taller stereocilium. The tension exerted on the tip of the lower stereocilium after the sound stimulation allows K<sup>+</sup> to enter the hair cells via the mechanoelectrical transducer (MET) channel on membranes of the lower stereocilium. Transmembrane channel like 1 and 2 (TMC1 and TMC2), tetraspan transmembrane protein hair cell stereocilia (LHFPL5), protocadherin-related 15 (PCDH15), and transmembrane inner ear (TMIE) proteins are likely to be involved in the organization of MET channels, although the channel's exact molecular

composition is not known. A *TMIE* mutation is associated with autosomal recessive nonsyndromic hearing loss, the most common form of congenitally acquired hearing impairment. *TMC1* variations are related to progressive postlingual hearing loss and profound prelingual deafness.

Loss-of-function mutations in several genes coding myosins and cell adhesion proteins are responsible for the development of the rare congenital disorder known as Usher syndrome. These include the molecular motor myosin VIIa (*MYO7A* also known as *USH1B*), cell-cell adhesion cadherin proteins *CDH23* (also known as *USH1D*) and *PCDH15* (also known as *USH1F*), the scaffold proteins USH1 protein network component sans (*USH1G*) and USH1 protein network component harmonin (*USH1C*) genes, and genes coding the proteins Usher syndrome 2A (*USH2A*) and deafness autosomal recessive 31 (*DFNB31* also known as *USH2D*). Functional alterations in the calcium and integrin binding family member 2 (*CIB2*) protein lead to the development of Usher syndrome type 1J and nonsyndromic deafness. And, finally, polymorphisms in *CDH23*, *PCDH15*, *MYO15A*, or *MYO6* predispose to age-related hearing loss (Ahmed et al., 2013; Azaiez et al., 2015; Brownstein et al., 2014; Cosgrove and Zallocchi, 2014; El-Amraoui and Petit, 2005; Hwang et al., 2012; Jiang et al., 2014; Kammerer et al., 2012; Kremer et al., 2006; Op de Beeck et al., 2011; Pan and Zhang, 2012; Reiners et al., 2006; Schwander et al., 2010; Verpy et al., 2011; Yan and Liu, 2010).

## II. Human disease pathways

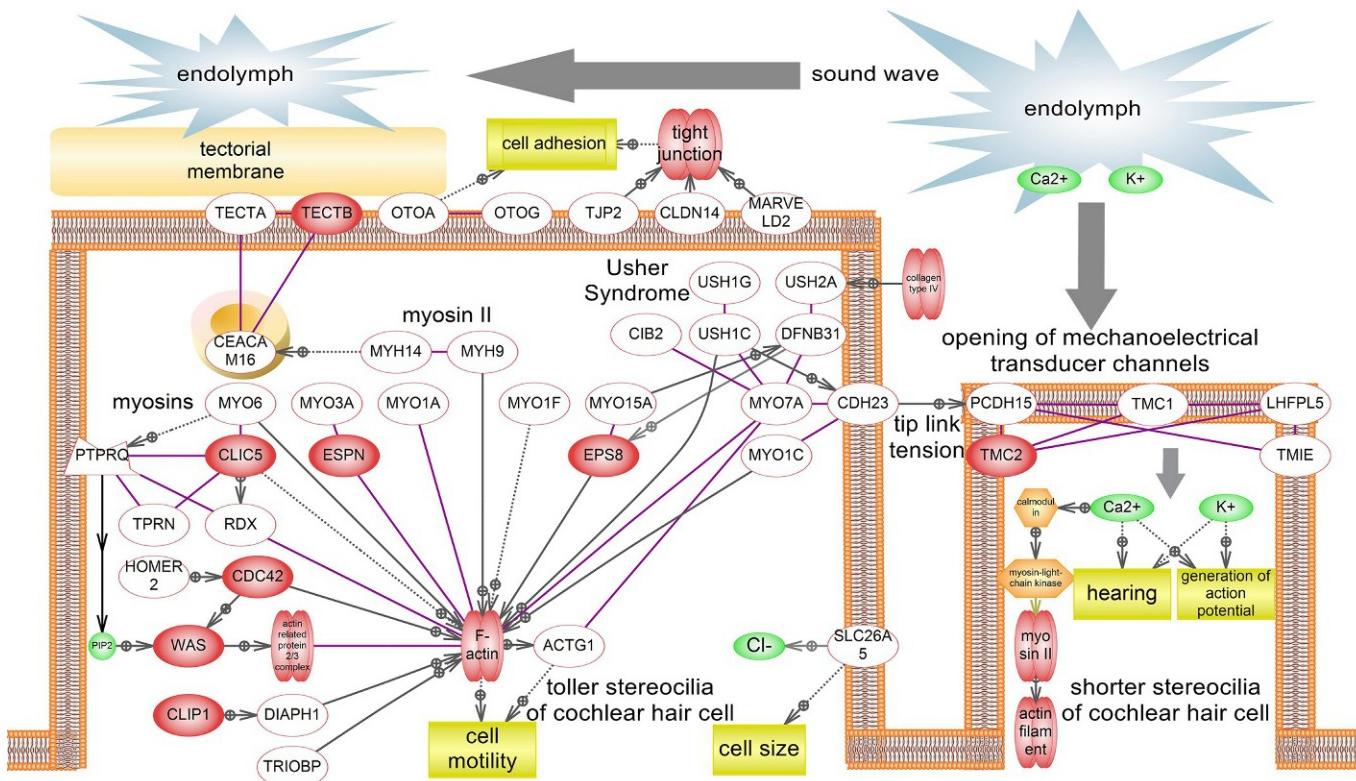


FIG. 1 Pathway 1: Dysfunction of cochlear hair cell stereocilia proteins in hearing loss.

## Pathway 2

### Dysfunctions of cochlear hair cell synapse proteins in hearing loss (Fig. 2)

#### Incoming signals

Hearing depends on neurotransmission from the cochlear hair cells to the peripheral axon of the spiral ganglion neuron through the glutamatergic synapse. Some genes, encoding proteins implicated in synaptogenesis, may be mutated and exhibit diminished functions in the congenital hearing loss. Those genes include otoferlin (*OTOF*), GIPC PDZ domain containing family member 3 (*GIPC3*), solute carrier family 17 (vesicular glutamate transporter), member 8 (*SLC17A8*), calcium voltage-gated channel subunit alpha1 D (*CACNA1D*), and myosin VI (*MYO6*).

#### Outcome effects

Due to dysfunctions of these proteins, the glutamatergic synapse between cochlear hair cells and peripheral axon of spiral ganglion neuron neurotransmission is impaired resulting in hearing loss (Charizopoulou et al., 2011; Cosgrove and Zallocchi, 2014; Friedman et al., 2009; Gregory et al., 2013; Heidrych et al., 2009; Luo et al., 2013; Moser et al., 2013; Newman et al., 2012; Pan and Zhang, 2012; Reiners et al., 2006; Roux et al., 2006; Yan and Liu, 2010; Zallocchi et al., 2012).

#### Signaling

The neurotransmitter glutamate needs to be loaded into synaptic vesicles before it is released into the synaptic cleft. The glutamatergic ribbon synapses of hair cells use the vesicular glutamate transporter *SLC17A8* (also known as VGLUT3) to load their synaptic vesicles with glutamate. Mutations in the *SLC17A8* gene cause autosomal dominant nonsyndromic deafness.

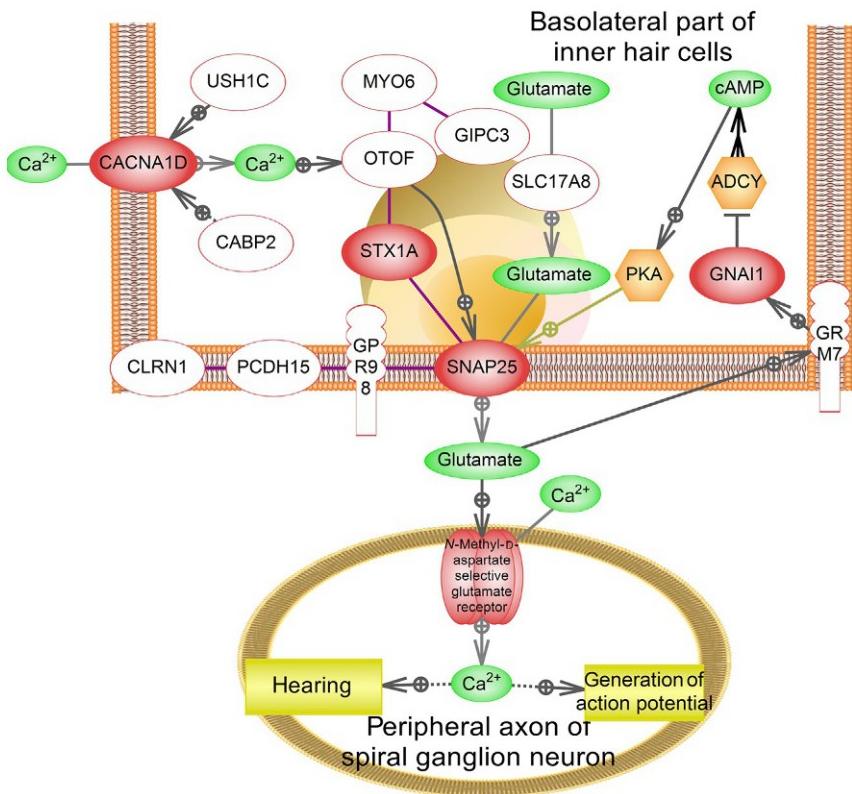
Unlike in other synapses, hair cell ribbon synapses use *CACNA1D* (CaV1.3 L-type  $\text{Ca}^{2+}$  channels) to stimulate glutamate secretion. The calcium-binding protein 2 (*CABP2*) might play a role in regulating *CACNA1D* and therefore inner hair cell synaptic transmission. A loss-of-function mutation in the *CACNA1D* gene has been linked to familial congenital deafness and bradycardia. Variations in the *CABP2* gene were associated with moderate sensorineural hearing impairment.

Mutations in *OTOF* cause both prelingual deafness and temperature-sensitive synaptic hearing impairment. *OTOF* binds  $\text{Ca}^{2+}$  during the hair cell glutamate exocytosis and may substitute for the classic synaptic fusion proteins synaptotagmins (SYT1 or SYT2). *OTOF*

supports  $\text{Ca}^{2+}$ -dependent interactions with syntaxin 1A (STX1A) and the synaptosome-associated protein 25 kDa (SNAP25). MYO6 was shown to be a novel OTOF-binding partner.

Mutations in other genes that play a role in vesicle exocytosis in cochlear hair cells have been associated with hearing loss. GIPC3 may take part in  $\text{Ca}^{2+}$ -dependent exocytosis in cochlear hair cells. PCDH15 and the adhesion G protein-coupled receptor V1 (ADGRV1) complex may connect with SNAP25 to control vesicle docking and fusion in synaptosomes from the organ of Corti. The absence or loss of function of one of the components of the complex results in a delay in synaptic maturation.

Finally, polymorphisms of the glutamate metabotropic receptor 7 (GRM7) gene are a significant risk factor for age-related hearing loss development. GRM7 activation inhibits the cyclic adenosine monophosphate (cAMP) cascade and synaptic glutamate exocytosis by providing negative feedback upon glutamate release. The lack of GRM7 function leads to neuronal damage due to glutamate excitotoxicity resulting in hearing loss.



**FIG. 2** Pathway 2: Dysfunction of cochlear hair cell synapse proteins in hearing loss.

## Pathway 3

### Impairment of mechanoelectrical transduction and potassium cycling in the inner ear in hearing loss (Fig. 3)

#### Incoming signals

The cochlear canals contain two types of fluid: perilymph and endolymph. Perilymph has an ionic composition similar to extracellular fluid found elsewhere in the body (i.e., it is K<sup>+</sup>-poor and Na<sup>+</sup>-rich), and it fills the scalae tympani and vestibule. Hearing depends on the high K<sup>+</sup> concentration in endolymph that bathes the apical membranes of sensory hair cells. K<sup>+</sup> enters the hair cell through mechanoelectrical transducer channels. K<sup>+</sup> ions exit from hair cells, transfer between endolymph and perilymph, and are recycled by Deiter cells, fibrocytes, and marginal cells of the stria vascularis. Dysfunctions in the proteins involved in mechanoelectrical transduction and K<sup>+</sup> recycling cause hearing loss.

#### Outcome effects

Dysfunctional proteins of mechanoelectrical transducer channel and K<sup>+</sup> channels impair the K<sup>+</sup> circulation in endolymph of the inner ear and the transduction of sound waves into neuronal signals normally produced by action potential generation in hair cell membrane.

#### Signaling

When stereocilia on cochlear hair cells move, mechanoelectrical transducer channels open, and K<sup>+</sup> enters the hair cell via apical MET channels. Mutations in the genes coding the MET channels (*TMC1*, *TMC2*, *LHFPL5*, *TMIE*, and *PCDH15*) are associated with different forms of congenital deafness (see [Pathway 1](#)).

When K<sup>+</sup> enters through the hair cell membrane, depolarization occurs. Depolarization in turn opens voltage-gated calcium channels (i.e., the purinergic receptor P2X 2 (P2RX2), transient receptor potential cation channel subfamily C member 1 (TRPC1), and the ATPase plasma membrane Ca<sup>2+</sup> transporting 1 and 2 (ATP2B2 and ATP2B2)) in the hair cell membrane to stimulate Ca<sup>2+</sup> influx and cause glutamate release from the basal end of the cell onto the auditory nerve endings (see [Pathway 2](#)). P2RX2 mutations are associated with autosomal dominant nonsyndromic hearing loss.

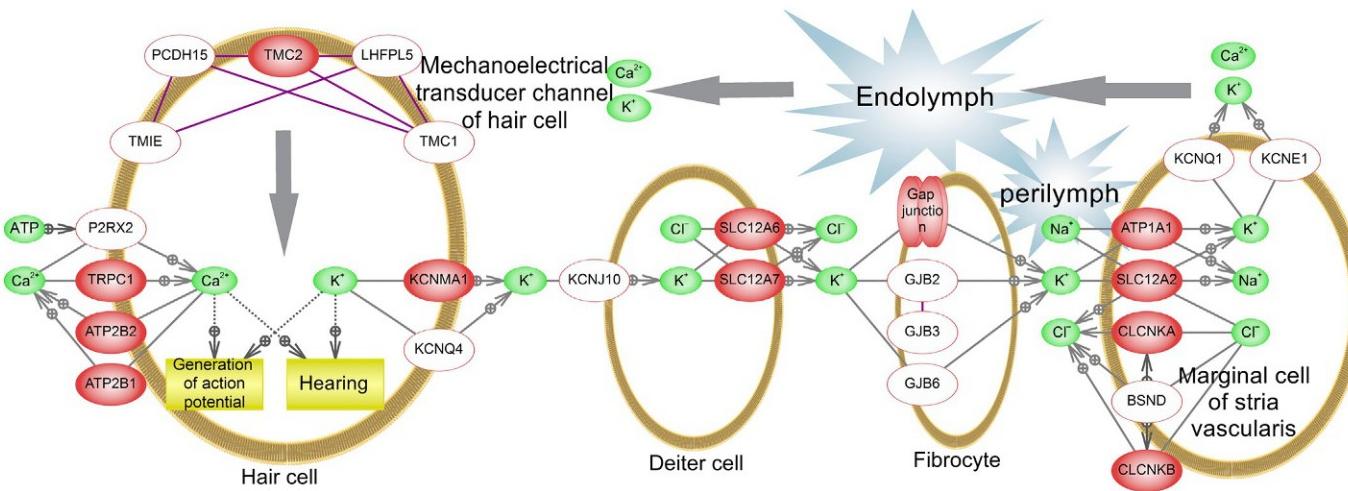
K<sup>+</sup> exits from the hair cells through the potassium voltage-gated channel subfamily Q member 4 (KCNQ4) and the potassium calcium-activated

channel subfamily M alpha 1 (KCNMA1) channels. KCNQ4 mutations are found in patients with nonsyndromic sensorineural deafness type 2, an autosomal dominant form of progressive hearing loss.

Supporting Deiters cells take K<sup>+</sup> back via potassium voltage-gated channel subfamily J member 10 (KCNJ10), and it is exported out by solute carrier family 12 (potassium/chloride transporters) and member VI and VII (SLC12A6 and SLC12A7). Mutations in KCNJ10 cause the autosomal recessive EAST syndrome characterized by epilepsy, ataxia, sensorineural deafness, and a salt-wasting tubulopathy. Polymorphisms in the KCNQ4 gene are strongly associated with several types of hearing loss including autosomal recessive EAST syndrome. The knockout of either the SLC12A6 or SLC12A7 genes causes deafness in mice.

K<sup>+</sup> passes between fibrocytes of the lateral wall through gap junctions. At least three connexin genes (gap junction protein beta GJB2, GJB3, and GJB6) belong to the gap junction system and are involved in congenital deafness. Mutations in GJB2 (the variation 35delG is the most common one) are responsible for as much as 50% of prelingual, recessive deafness.

In stria vascularis marginal cells, the solute carrier family 12 (sodium/potassium/chloride transporter) member 2 (SLC12A2 also known as NKCC1), ATPase Na<sup>+</sup>/K<sup>+</sup> transporting subunit alpha 1 and 2 (ATP1A1 and ATP1A2) raise the intracellular K<sup>+</sup> concentration. In parallel the chloride voltage-gated channel Ka/barttin CLCNK type accessory beta subunit (CLCNKA/BSND) and chloride voltage-gated channel Kb/barttin CLCNK type accessory beta subunit (CLCNKB/BSND) channels recycle Cl<sup>-</sup>. BSND is thought to be an accessory subunit of a chloride channel, and if mutated, it disrupts the activity of CLCNKA and CLCNKB. Mutations in the BSND gene are associated with Bartter syndrome leading to sensorineural deafness (Bartter syndrome type IV). Further, K<sup>+</sup> exits through apical channels, specifically the potassium voltage-gated channel subfamily Q member 1 (KCNQ1) and potassium voltage-gated channel subfamily E regulatory subunit 1 (KCNE1) back into the endolymph. Mutations in the KCNE1 and KCNQ1 genes cause Jervell and Lange-Nielsen syndrome (long QT syndrome, associated with a bilateral sensorineural hearing loss). Interestingly, SLC12A2, ATP1A1, and ATP1A2 heterozygous deletions were shown to cause an age-dependent hearing loss in mice (Chen and Zhao, 2014; Hibino and Kurachi, 2006; Janssen et al., 2009; Lang et al., 2007; Mahdieh and Rabbani, 2009; Naito et al., 2013; Nie, 2008; Sliwinska-Kowalska and Pawelczyk, 2013; Tian et al., 2007; Van Eyken et al., 2006, 2007; Wang et al., 2014; Zhang et al., 2014).



**FIG. 3** Pathway 3: Deficiency of potassium cycling in hearing loss.

## References

- Disease numbers # 609533, # 601067, # 601386, # 612976, # 606346, # 601071 (and many others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code H90/H91/H91.9. Diseases of the ear and mastoid process (H60-H95). (ICD-10, <https://icdlist.com>). ICD-11: disease code AB56/AB50/AB51.
- Ahmed, Z.M., Frolenkov, G.I., Riazuddin, S., 2013. Usher proteins in inner ear structure and function. *Physiol. Genomics* 45, 987–989. <https://doi.org/10.1152/physiolgenomics.00135.2013>.
- Azaiez, H., Decker, A.R., Booth, K.T., Simpson, A.C., Shearer, A.E., Huygen, P.L.M., Bu, F., Hildebrand, M.S., Ranum, P.T., Shibata, S.B., Turner, A., Zhang, Y., Kimberling, W.J., Cornell, R.A., Smith, R.J.H., 2015. HOMER2, a stereociliary scaffolding protein, is essential for normal hearing in humans and mice. *PLoS Genet.* 11, e1005137. <https://doi.org/10.1371/journal.pgen.1005137>.
- Brownstein, Z., Abu-Rayyan, A., Karfunkel-Doron, D., Sirigu, S., Davidov, B., Shohat, M., Frydman, M., Houdusse, A., Kanaan, M., Avraham, K.B., 2014. Novel myosin mutations for hereditary hearing loss revealed by targeted genomic capture and massively parallel sequencing. *Eur. J. Hum. Genet.* 22, 768–775. <https://doi.org/10.1038/ejhg.2013.232>.
- Charizopoulou, N., Lelli, A., Schraders, M., Ray, K., Hildebrand, M.S., Ramesh, A., Srisailapathy, C.R.S., Oostrik, J., Admiraal, R.J.C., Neely, H.R., Latache, J.R., Smith, R.J.H., Northup, J.K., Kremer, H., Holt, J.R., Noben-Trauth, K., 2011. Gipc3 mutations associated with audiogenic seizures and sensorineural hearing loss in mouse and human. *Nat. Commun.* 2, 201. <https://doi.org/10.1038/ncomms1200>.
- Chen, J., Zhao, H.-B., 2014. The role of an inwardly rectifying K<sup>(+)</sup> channel (Kir4.1) in the inner ear and hearing loss. *Neuroscience* 265, 137–146. <https://doi.org/10.1016/j.neuroscience.2014.01.036>.
- Cosgrove, D., Zallocchi, M., 2014. Usher protein functions in hair cells and photoreceptors. *Int. J. Biochem. Cell Biol.* 46, 80–89. <https://doi.org/10.1016/j.biocel.2013.11.001>.
- El-Amraoui, A., Petit, C., 2005. Usher I syndrome: unravelling the mechanisms that underlie the cohesion of the growing hair bundle in inner ear sensory cells. *J. Cell Sci.* 118, 4593–4603. <https://doi.org/10.1242/jcs.02636>.
- Friedman, R.A., Van Laer, L., Huentelman, M.J., Sheth, S.S., Van Eyken, E., Corneveaux, J.J., Tembe, W.D., Halperin, R.F., Thorburn, A.Q., Thys, S., Bonneux, S., Fransen, E., Huyghe, J., Pyykkö, I., Cremers, C.W.R.J., Kremer, H., Dhooge, I., Stephens, D., Orzan, E., Pfister, M., Bille, M., Parving, A., Sorri, M., Van de Heyning, P.H., Makmura, L., Ohmen, J.D., Linthicum, F.H., Fayad, J.N., Pearson, J.V., Craig, D.W., Stephan, D.A., Van Camp, G., 2009. GRM7 variants confer susceptibility to age-related hearing impairment. *Hum. Mol. Genet.* 18, 785–796. <https://doi.org/10.1093/hmg/ddn402>.
- Gregory, F.D., Pangrsic, T., Calin-Jageman, I.E., Moser, T., Lee, A., 2013. Harmonin enhances voltage-dependent facilitation of Cav1.3 channels and synchronous exocytosis in mouse inner hair cells. *J. Physiol.* 591, 3253–3269. <https://doi.org/10.1113/jphysiol.2013.254367>.
- Heidrych, P., Zimmermann, U., Kuhn, S., Franz, C., Engel, J., Duncker, S.V., Hirt, B., Pusch, C.M., Ruth, P., Pfister, M., Marcotti, W., Blin, N., Knipper, M., 2009. Otoferlin interacts with myosin VI: implications for maintenance of the basolateral synaptic structure of the inner hair cell. *Hum. Mol. Genet.* 18, 2779–2790. <https://doi.org/10.1093/hmg/ddp213>.
- Hibino, H., Kurachi, Y., 2006. Molecular and physiological bases of the K<sup>+</sup> circulation in the mammalian inner ear. *Physiology* 21, 336–345. <https://doi.org/10.1152/physiol.00023.2006>.
- Hwang, J.-H., Liu, K.S., Wu, C.-C., Liu, T.-C., 2012. Association of cadherin23 single nucleotide polymorphism with age-related hearing impairment in Han Chinese. *Otolaryngol. Head Neck Surg. Off. J. Am. Acad.* 147, 531–534. <https://doi.org/10.1177/0194599812446904>.
- Janssen, A.G.H., Scholl, U., Domeyer, C., Nothmann, D., Leinenweber, A., Fahlke, C., 2009. Disease-causing dysfunctions of barttin in Bartter syndrome type IV. *J. Am. Soc. Nephrol.* 20, 145–153. <https://doi.org/10.1681/ASN.2008010102>.

- Jiang, L., Phang, J.M., Yu, J., Harrop, S.J., Sokolova, A.V., Duff, A.P., Wilk, K.E., Alkhamici, H., Breit, S.N., Valenzuela, S.M., Brown, L.J., Curmi, P.M.G., 2014. CLIC proteins, ezrin, radixin, moesin and the coupling of membranes to the actin cytoskeleton: a smoking gun? *Biochim. Biophys. Acta* 1838, 643–657. <https://doi.org/10.1016/j.bbamem.2013.05.025>.
- Kammerer, R., Rüttiger, L., Riesenberger, R., Schäuble, C., Krupar, R., Kamp, A., Sunami, K., Eisenried, A., Hennenberg, M., Grunert, F., Bress, A., Battaglia, S., Schrewe, H., Knipper, M., Schneider, M.R., Zimmermann, W., 2012. Loss of mammal-specific tectorial membrane component carcinoembryonic antigen cell adhesion molecule 16 (CEACAM16) leads to hearing impairment at low and high frequencies. *J. Biol. Chem.* 287, 21584–21598. <https://doi.org/10.1074/jbc.M111.320481>.
- Kremer, H., van Wijk, E., Märker, T., Wolfrum, U., Roepman, R., 2006. Usher syndrome: molecular links of pathogenesis, proteins and pathways. *Hum. Mol. Genet.* 15 (2), R262–R270. <https://doi.org/10.1093/hmg/ddl205>.
- Lang, F., Vallon, V., Knipper, M., Wangemann, P., 2007. Functional significance of channels and transporters expressed in the inner ear and kidney. *Am. J. Physiol. Cell Physiol.* 293, C1187–C1208. <https://doi.org/10.1152/ajpcell.00024.2007>.
- Luo, H., Yang, T., Jin, X., Pang, X., Li, J., Chai, Y., Li, L., Zhang, Y., Zhang, L., Zhang, Z., Wu, W., Zhang, Q., Hu, X., Sun, J., Jiang, X., Fan, Z., Huang, Z., Wu, H., 2013. Association of GRM7 variants with different phenotype patterns of age-related hearing impairment in an elderly male Han Chinese population. *PLoS One* 8, e77153. <https://doi.org/10.1371/journal.pone.0077153>.
- Mahdieh, N., Rabbani, B., 2009. Statistical study of 35delG mutation of GJB2 gene: a meta-analysis of carrier frequency. *Int. J. Audiol.* 48, 363–370. <https://doi.org/10.1080/14992020802607449>.
- Moser, T., Predoehl, F., Starr, A., 2013. Review of hair cell synapse defects in sensorineural hearing impairment. *Otol. Neurotol. Off. Publ. Am. Otol. Soc. Am. Neurotol. Soc. Eur. Acad. Otol. Neurotol.* 34, 995–1004. <https://doi.org/10.1097/MAO.0b013e3182814d4a>.
- Naito, T., Nishio, S., Iwasa, Y., Yano, T., Kumakawa, K., Abe, S., Ishikawa, K., Kojima, H., Namba, A., Oshikawa, C., Usami, S., 2013. Comprehensive genetic screening of KCNQ4 in a large autosomal dominant nonsyndromic hearing loss cohort: genotype-phenotype correlations and a founder mutation. *PLoS One* 8, e63231. <https://doi.org/10.1371/journal.pone.0063231>.
- Newman, D.L., Fisher, L.M., Ohmen, J., Parody, R., Fong, C.-T., Frisina, S.T., Mapes, F., Eddins, D.A., Robert Frisina, D., Frisina, R.D., Friedman, R.A., 2012. GRM7 variants associated with age-related hearing loss based on auditory perception. *Hear. Res.* 294, 125–132. <https://doi.org/10.1016/j.heares.2012.08.016>.
- Nie, L., 2008. KCNQ4 mutations associated with nonsyndromic progressive sensorineural hearing loss. *Curr. Opin. Otolaryngol. Head Neck Surg.* 16, 441–444. <https://doi.org/10.1097/MOO.0b013e32830f4aa3>.
- Op de Beeck, K., Schacht, J., Van Camp, G., 2011. Apoptosis in acquired and genetic hearing impairment: the programmed death of the hair cell. *Hear. Res.* 281, 18–27. <https://doi.org/10.1016/j.heares.2011.07.002>.
- Pan, L., Zhang, M., 2012. Structures of usher syndrome 1 proteins and their complexes. *Physiology* 27, 25–42. <https://doi.org/10.1152/physiol.00037.2011>.
- Reiners, J., Nagel-Wolfrum, K., Jürgens, K., Märker, T., Wolfrum, U., 2006. Molecular basis of human Usher syndrome: deciphering the meshes of the Usher protein network provides insights into the pathomechanisms of the Usher disease. *Exp. Eye Res.* 83, 97–119. <https://doi.org/10.1016/j.exer.2005.11.010>.
- Roux, I., Safieddine, S., Nouvian, R., Grati, M., Simmler, M.-C., Bahloul, A., Perfettini, I., Le Gall, M., Rostaing, P., Hamard, G., Triller, A., Avan, P., Moser, T., Petit, C., 2006. Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* 127, 277–289. <https://doi.org/10.1016/j.cell.2006.08.040>.

- Schwander, M., Kachar, B., Müller, U., 2010. Review series: the cell biology of hearing. *J. Cell Biol.* 190, 9–20. <https://doi.org/10.1083/jcb.201001138>.
- Sliwinska-Kowalska, M., Pawelczyk, M., 2013. Contribution of genetic factors to noise-induced hearing loss: a human studies review. *Mutat. Res.* 752, 61–65. <https://doi.org/10.1016/j.mrrev.2012.11.001>.
- Tian, C., Vanoye, C.G., Kang, C., Welch, R.C., Kim, H.J., George, A.L., Sanders, C.R., 2007. Preparation, functional characterization, and NMR studies of human KCNE1, a voltage-gated potassium channel accessory subunit associated with deafness and long QT syndrome. *Biochemistry* 46, 11459–11472. <https://doi.org/10.1021/bi700705j>.
- Van Eyken, E., Van Laer, L., Fransen, E., Topsakal, V., Lemkens, N., Laureys, W., Nelissen, N., Vandeveldé, A., Wienker, T., Van De Heyning, P., Van Camp, G., 2006. KCNQ4: a gene for age-related hearing impairment? *Hum. Mutat.* 27, 1007–1016. <https://doi.org/10.1002/humu.20375>.
- Van Eyken, E., Van Laer, L., Fransen, E., Topsakal, V., Hendrickx, J.-J., Demeester, K., Van de Heyning, P., Mäki-Torkko, E., Hannula, S., Sorri, M., Jensen, M., Parving, A., Bille, M., Baur, M., Pfister, M., Bonaconsa, A., Mazzoli, M., Orzan, E., Espeso, A., Stephens, D., Verbruggen, K., Huyghe, J., Dhooge, I., Huygen, P., Kremer, H., Cremers, C., Kunst, S., Manninen, M., Pyykkö, I., Rajkowska, E., Pawelczyk, M., Sliwinska-Kowalska, M., Steffens, M., Wienker, T., Van Camp, G., 2007. The contribution of GJB2 (Connexin 26) 35delG to age-related hearing impairment and noise-induced hearing loss. *Otol. Neurotol.* Off. Publ. Am. Otol. Soc. Am. Neurotol. Soc. Eur. Acad. Otol. Neurotol. 28, 970–975. <https://doi.org/10.197/MAO.0b013e3180dca1b9>.
- Verpy, E., Leibovici, M., Michalski, N., Goodyear, R.J., Houdon, C., Weil, D., Richardson, G.P., Petit, C., 2011. Stereocilin connects outer hair cell stereocilia to one another and to the tectorial membrane. *J. Comp. Neurol.* 519, 194–210. <https://doi.org/10.1002/cne.22509>.
- Wang, H., Zhao, Y., Yi, Y., Gao, Y., Liu, Q., Wang, D., Li, Q., Lan, L., Li, N., Guan, J., Yin, Z., Han, B., Zhao, F., Zong, L., Xiong, W., Yu, L., Song, L., Yi, X., Yang, L., Petit, C., Wang, Q., 2014. Targeted high-throughput sequencing identifies pathogenic mutations in KCNQ4 in two large Chinese families with autosomal dominant hearing loss. *PLoS One* 9, e103133. <https://doi.org/10.1371/journal.pone.0103133>.
- Yan, D., Liu, X.Z., 2010. Genetics and pathological mechanisms of Usher syndrome. *J. Hum. Genet.* 55, 327–335. <https://doi.org/10.1038/jhg.2010.29>.
- Zallocchi, M., Meehan, D.T., Delimont, D., Rutledge, J., Gratton, M.A., Flannery, J., Cosgrove, D., 2012. Role for a novel Usher protein complex in hair cell synaptic maturation. *PLoS One* 7, e30573. <https://doi.org/10.1371/journal.pone.0030573>.
- Zhang, X., Liu, Y., Zhang, L., Yang, Z., Shao, Y., Jiang, C., Wang, Q., Fang, X., Xu, Y., Wang, H., Zhang, S., Zhu, Y., 2014. Genetic variations in protocadherin 15 and their interactions with noise exposure associated with noise-induced hearing loss in Chinese population. *Environ. Res.* 135, 247–252. <https://doi.org/10.1016/j.envres.2014.09.021>.

## CHAPTER

## 7.2

## Otitis media

Otitis media is defined as an infection of the middle ear fluid and is the second most common pediatric diagnosis in the emergency department following upper respiratory infections. Although otitis media can occur at any age, it is most commonly seen between the ages of 6–24 months.

Otitis media is the rapid onset of signs and symptoms of inflammation in the middle ear. (*Ferri and Ferri, 2018*).

Infection of the middle ear can be viral, bacterial or a coinfection with both. The most common etiologic factor is a viral upper respiratory tract infection, which causes inflammation and dysfunction of the eustachian tube leading to the transient aspiration of nasopharyngeal secretions into the middle ear. The most common viral pathogens of otitis media include the respiratory syncytial virus (RSV), coronaviruses, influenza viruses, adenoviruses, human metapneumovirus, and picornaviruses ([Danishyar and Ashurst, 2018](#)). Bacterial colonization from the nasopharynx in conjunction with eustachian tube dysfunction also leads to the infection. The most common bacteria that cause otitis media are *Streptococcus pneumoniae* (*S. pneumoniae*), followed by Nontypeable *Haemophilus influenzae* (NTHi) and *Moraxella catarrhalis*. *S. pneumoniae* causes from 30% to 40% of all cases of otitis media. The second most common bacterial pathogen is *H. influenzae*, which causes up to 50% of cases. *M. catarrhalis* causes the last proportion of 10%–20% of cases. Infection caused by penicillin-nonsusceptible *S. pneumoniae* (PNSSP) ( $\text{MIC} > 0.1 \text{ mg/mL}$ ) becomes the infection of increasing importance ranging from 8% to 34% of all otitis media cases. About 50% of PNSSP isolates are penicillin intermediate (with MIC of 0.1–2.0 mg/mL) (*Ferri and Ferri, 2018*).

The epithelial cells of the middle ear contain several defense mechanisms including (1) the presence of mucous glycoproteins and surfactants, which trap infectious agents; (2) the ability to secrete defense molecules such as the defensins or interferons; and (3) increased antibody production through the adaptive immune response.

The low level of activity of Toll-like receptor (TLR) signaling in epithelial cells in the human middle ear decreases the secretion of defense

molecules and cytokines by epithelial cells, which in turn is needed for activation of cells of immune system:

**Pathway 1.** *Insufficient activation of immune response in the middle ear epithelium cells in otitis media (Fig. 4).*

Pathogens also stimulate extra mucus production in the middle ear, however, which further complicate the reduction of inflammation characteristic of otitis media.

**Pathway 2.** *Pathogens stimulate mucins expression in the middle ear (Fig. 5).*

## Key cellular contributors and processes

Extracellular matrix proteins

Protein or gene

The extracellular matrix (ECM), an essential component of most tissues in multicellular organisms, is a noncellular network of macromolecules secreted by the surrounding cells. The ECM provides structural support to the tissue and is strongly involved in intercellular signaling.

Middle ear

Anatomic structure

Middle ear is the internal part of the ear that conducts sound from the outer to the inner ear.

Mucus

Process

Mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may tether together at the fluid surface by oligomeric mucin protein complexes.

NOD-like receptors

Protein or gene

The NOD-like receptors (nucleotide-binding oligomerization domain-like receptors, NLRs) are cytoplasmic pattern recognition receptors. NLRs can bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) inside the cell and have a variety of functions in the regulation of inflammatory and apoptotic responses. The NLR family consists of several proteins divided into subfamilies based on their N-terminal protein-interacting domains.

Toll-like receptors

Protein or gene

Toll-like receptors belong to a family of membrane proteins that can directly bind microbial molecules or proteins and initiate the innate immune response.

## Pathway 1

### **Insufficient activation of immune response in the middle ear epithelium cells in otitis media ([Fig. 4](#))**

#### **Incoming signals**

Low expression of pattern recognition receptors leads to insufficient immune response in the middle ear epithelium cells in otitis media. Middle ear epithelial cells express all types of pattern recognition receptors such as the Toll-like receptors (TLRs), cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors, C-type lectin receptors, and retinoic acid-inducible genes (*DDX58* (DExD/H-box helicase 58)). TLR signaling provides protection against infection by recognizing intruding pathogens through their invariant pathogen-associated molecular patterns and mobilizing appropriate immune system response. Patients with chronic middle ear disease have been shown to exhibit lower mRNA and protein levels for TLR2, TLR4, TLR5, TLR7, and TLR9 compared with a control group.

#### **Outcome effects**

The downregulation of TLRs, NODs, and other pattern recognition receptor expression in otitis media leads to an inefficient defense in the middle ear, which in turn causes repeated infections and persistent inflammations.

#### **Signaling**

TLRs can sense pathogens through their pathogen-associated molecular patterns. Among others, TLR3 recognizes dsRNA, TLR2 and TLR4 recognize bacterial lipopolysaccharides (LPS), TLR5 responds to bacterial flagellin, TLR7/8 mediates recognition of ssRNA, and TLR9 recognizes the CpG sites of bacterial and viral DNA. Also, proteins derived from *H. influenzae* serve as ligands for TLR2 in otitis media. A lipooligosaccharide (LOS), which is expressed on mucosal Gram-negative bacteria, serves as a ligand for both TLR2 and TLR4. Proteins specific for *S. pneumoniae* are considered ligands for TLR4.

The activation of most TLRs results in downstream activation of the myeloid differentiation primary response 88 (MyD88) gene, which in turn activates the interleukin 1 receptor-associated kinase (IRAK1–IRAK4) and TNF receptor-associated factor 6 (TRAF6) cascades. Then, activation of MAPKs and transcription factors (primary NF- $\kappa$ B and JUN/FOS) occurs

leading to the expression of proinflammatory proteins and the stimulation of immune responses.

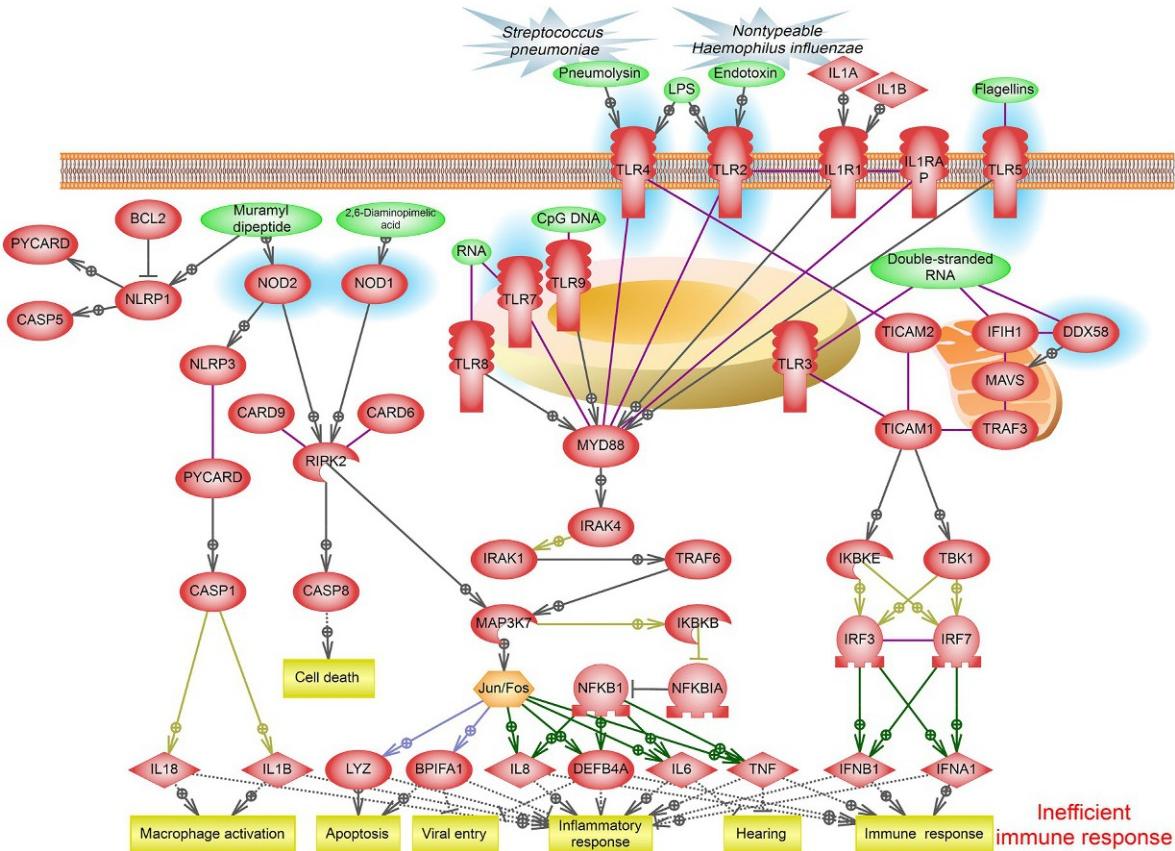
When pathogens bypass the membrane-associated pattern recognition receptors, they encounter cytoplasmic pattern recognition receptors such as the nucleotide-binding oligomerization domain containing 1,2 (NOD1,2), interferon induced with helicase C domain 1 (IFIH1), and DExD/H-box helicase (58DDX58) proteins. NOD1 and NOD2 initiate immune responses through the formation of inflammasomes, and they activate NF- $\kappa$ B, leading to the production of inflammatory cytokines. Patients with otitis media have significantly lower levels of expression of NOD1 and NOD2, as well as DDX58. The development of recurrent otitis media may be associated with these decreased expression levels, demonstrating the protective roles of NOD1, NOD2, and DDX58 against ear infections.

As a result of insufficient activation, TLR and NOD signaling in middle ear epithelium does not promote the release of enough cytokines, interferons, and other defensive proteins.

For example, BPI fold containing family A member 1 (BPIFA1) and DEFB4A (human beta-defensin 2) have broad-spectrum antimicrobial activity. They reduce bacterial biofilm formation by *Pseudomonas aeruginosa*. BPIFA1 also acts as a chemoattractant that recruits macrophages and neutrophils to the site of infection. It has been found that BPIFA1 is essential in the maintenance of middle ear fluid pressure and efficient mucociliary clearance.

Middle ear epithelial cells also produce lysozyme, an antimicrobial molecule of innate immunity that degrades the peptidoglycans found in bacterial cell walls. Lysozyme and DEFB4A have synergistic effects against *S. pneumoniae* in otitis media (Chen et al., 2004; Granath et al., 2011; Hirano et al., 2007; Kim et al., 2010, 2014; Lee et al., 2008, 2013; Mittal et al., 2014; Moon et al., 2006; Philpott et al., 2014; Shimada et al., 2008; Si et al., 2014).

## II. Human disease pathways



**FIG. 4** Pathway 1: Insufficient activation of immune response in the middle ear epithelium cells in otitis media.

## Pathway 2

### Pathogens stimulate mucins expression in the middle ear (Fig. 5)

#### Incoming signals

The Gram-positive bacterium *S. pneumoniae*, Gram-negative bacteria nontypable *H. influenza* (NTHi) and *M. catarrhalis* synergistically induce the activation of mucus production in the middle ear effusion of patients with chronic otitis media. The viscous mucus of the middle ear is a heterogeneous mixture of secreted polypeptides, mainly mucins. The rise of mucin production is a vital defense response against invading microbes (also see Asthma). Excess mucin production, however, results in a conductive hearing loss observed in otitis media.

#### Outcome effects

Abnormally generous amount of viscous mucus in the middle ear prevents active mucociliary clearance in otitis media. The overproduction of MUC2 (mucin 2, oligomeric mucus/gel-forming), MUC5AC (mucin 5AC), and MUC5B (mucin 5B) by epithelial cells obstructs the transmission of sound waves from the middle ear to the inner ear.

#### Signaling

Pathogens adhered to host epithelial cells stimulate the activation of TRL pathways. Polymorphisms in the gene encoding *TLR4* have been associated with recurrent acute otitis media. Pneumolysin, endotoxin, and lipopolysaccharides are typical trigger signals produced by *S. pneumoniae*, NTHi, and *M. catarrhalis*, respectively. These ligands induce mucin (MUC5AC, MUC5B, and MUC2) expression through the activation of the MyD88-MAP3K7 and MAP3K1 (mitogen-activated protein kinase kinase kinase 7 and 1) cascade. The activation of MAPKs is also required for the synergistic induction of mucin expression by pathogens.

Also, *S. pneumoniae* works synergistically with NTHi to induce mucin expression via an AP1-dependent mechanism. Gram-negative NTHi and *S. pneumoniae* synergistically induce activation of major AP-1 subunits including activating transcription factor 2 (ATF-2) and JUN (Jun proto-oncogene, AP-1 transcription factor subunit).

Epidermal growth factor receptor (EGFR) signaling is also involved in the activation of the JUN and FOS (Fos proto-oncogene, an AP-1 transcription factor subunit) transcription factors and leads to mucin synthesis.

Interleukin-1B (IL-1B) and tumor necrosis factor (TNF) signals stimulate mucin expression via canonical NF-kb activation. The canonical NF-kb

pathway is initiated by TNF via its cognate receptor (TNFR1) and by IL-1 via the IL-1 receptor (IL-1R) (Bhutta et al., 2017; Cho et al., 2016; Elsheikh and Mahfouz, 2006; Emonts et al., 2007; Ha et al., 2008; Hernandez et al., 2015; Kawano et al., 2000; Kerschner, 2007; Kerschner et al., 2010; Leichtle et al., 2009; MacArthur et al., 2011; Preciado et al., 2010; Shen et al., 2008; Ubell et al., 2008).

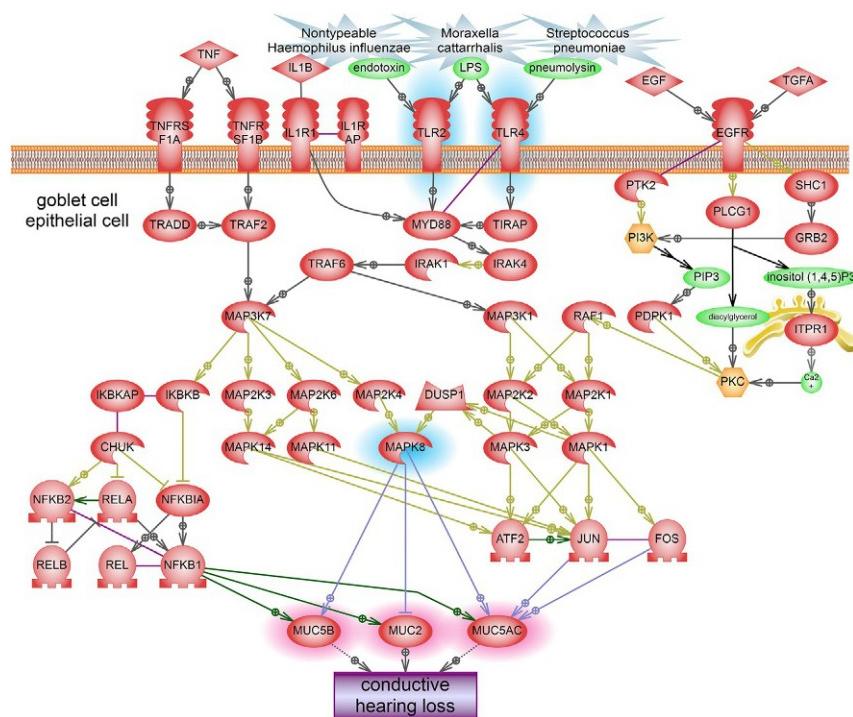


FIG. 5 Pathway 2: Pathogens stimulate mucins expression in the middle ear.

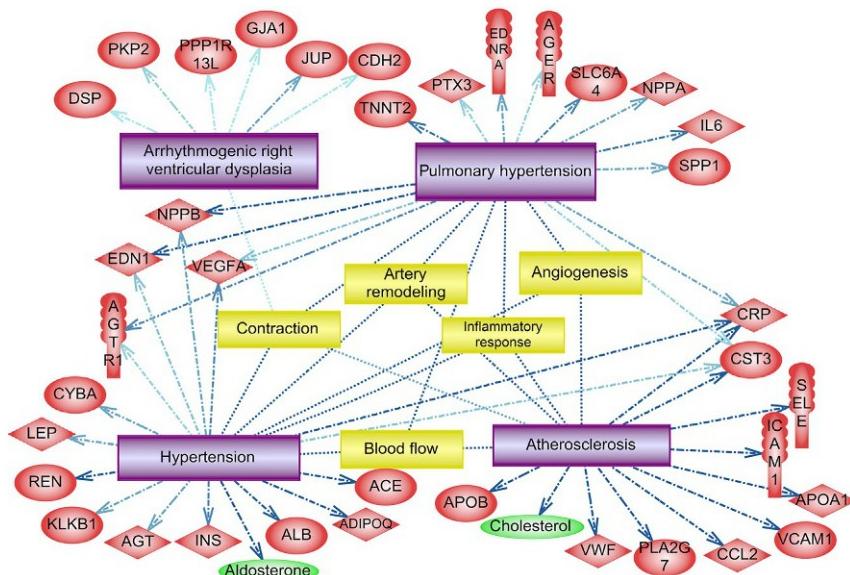
## References

- Disease numbers # 166760 (and many others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code H65-H67. Diseases of the ear and mastoid process (H60-H95). (ICD-10, <https://icdlist.com>). ICD-11: disease code AB0Z/AB00/AB01/AB0Y.
- Bhutta, M.F., Thornton, R.B., Kirkham, L.-A.S., Kerschner, J.E., Cheeseman, M.T., 2017. Understanding the aetiology and resolution of chronic otitis media from animal and human studies. *Dis. Model. Mech.* 10, 1289–1300. <https://doi.org/10.1242/dmm.029983>.
- Chen, R., Lim, J.H., Jono, H., Gu, X.-X., Kim, Y.S., Basbaum, C.B., Murphy, T.F., Li, J.-D., 2004. Nontypeable *Haemophilus influenzae* lipoprotein P6 induces MUC5AC mucin transcription via TLR2-TAK1-dependent p38 MAPK-AP1 and IKK $\beta$ -IkappaB $\alpha$ -NF- $\kappa$ B signaling pathways. *Biochem. Biophys. Res. Commun.* 324, 1087–1094. <https://doi.org/10.1016/j.bbrc.2004.09.157>.
- Cho, C.G., Pak, K., Webster, N., Kurabi, A., Ryan, A.F., 2016. Both canonical and non-canonical NF- $\kappa$ B activation contribute to the proliferative response of the middle ear mucosa during bacterial infection. *Innate Immun.* 22, 626–634. <https://doi.org/10.1177/1753425916668581>.
- Danishyar, A., Ashurst, J.V., 2018. Otitis, media, acute. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Elsheikh, M.N., Mahfouz, M.E., 2006. Up-regulation of MUC5AC and MUC5B mucin genes in nasopharyngeal respiratory mucosa and selective up-regulation of MUC5B in middle ear in pediatric otitis media with effusion. *Laryngoscope* 116, 365–369. <https://doi.org/10.1097/01.MLG.0000195290.71090.A1>.
- Emonts, M., Veenhoven, R.H., Wiertsema, S.P., Houwing-Duistermaat, J.J., Walraven, V., de Groot, R., Hermans, P.W.M., Sanders, E.A.M., 2007. Genetic polymorphisms in immunoresponse genes TNFA, IL6, IL10, and TLR4 are associated with recurrent acute otitis media. *Pediatrics* 120, 814–823. <https://doi.org/10.1542/peds.2007-0524>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Granath, A., Cardell, L.-O., Uddman, R., Harder, H., 2011. Altered Toll- and Nod-like receptor expression in human middle ear mucosa from patients with chronic middle ear disease. *J. Inf. Secur.* 63, 174–176. <https://doi.org/10.1016/j.jinf.2011.06.006>.
- Ha, U.-H., Lim, J.H., Kim, H.-J., Wu, W., Jin, S., Xu, H., Li, J.-D., 2008. MKP1 regulates the induction of MUC5AC mucin by *Streptococcus pneumoniae* pneumolysin by inhibiting the PAK4-JNK signaling pathway. *J. Biol. Chem.* 283, 30624–30631. <https://doi.org/10.1074/jbc.M802519200>.
- Hernandez, M., Leichtle, A., Pak, K., Webster, N.J., Wasserman, S.I., Ryan, A.F., 2015. The transcriptome of a complete episode of acute otitis media. *BMC Genomics* 16, 259. <https://doi.org/10.1186/s12864-015-1475-7>.
- Hirano, T., Kodama, S., Fujita, K., Maeda, K., Suzuki, M., 2007. Role of Toll-like receptor 4 in innate immune responses in a mouse model of acute otitis media. *FEMS Immunol. Med. Microbiol.* 49, 75–83. <https://doi.org/10.1111/j.1574-695X.2006.00186.x>.
- Kawano, H., Paparella, M.M., Ho, S.B., Schachern, P.A., Morizono, N., Le, C.T., Lin, J., 2000. Identification of MUC5B mucin gene in human middle ear with chronic otitis media. *Laryngoscope* 110, 668–673. <https://doi.org/10.1097/00005537-200004000-00024>.
- Kerschner, J.E., 2007. Mucin gene expression in human middle ear epithelium. *Laryngoscope* 117, 1666–1676. <https://doi.org/10.1097/MLG.0b013e31806db531>.
- Kerschner, J.E., Tripathi, S., Khampang, P., Papsin, B.C., 2010. MUC5AC expression in human middle ear epithelium of patients with otitis media. *Arch. Otolaryngol. Head Neck Surg.* 136, 819–824. <https://doi.org/10.1001/archoto.2010.123>.

- Kim, M.G., Park, D.C., Shim, J.S., Jung, H., Park, M.S., Kim, Y.I., Lee, J.W., Yeo, S.G., 2010. TLR-9, NOD-1, NOD-2, RIG-I and immunoglobulins in recurrent otitis media with effusion. *Int. J. Pediatr. Otorhinolaryngol.* 74, 1425–1429. <https://doi.org/10.1016/j.ijporl.2010.09.026>.
- Kim, Y.J., Cha, S.H., Lee, H.Y., Lee, S.K., Chung, H.Y., Yeo, J.H., Kim, Y.I., Yeo, S.G., 2014. Decreased pattern-recognition receptor-mediated cytokine mRNA expression in obese children with otitis media with effusion. *Clin. Exp. Otorhinolaryngol.* 7, 7–12. <https://doi.org/10.3342/ceo.2014.7.1.7>.
- Lee, H.-Y., Takeshita, T., Shimada, J., Akopyan, A., Woo, J.-I., Pan, H., Moon, S.K., Andalibi, A., Park, R.-K., Kang, S.-H., Kang, S.-S., Gellibolian, R., Lim, D.J., 2008. Induction of beta defensin 2 by NTTHi requires TLR2 mediated MyD88 and IRAK-TRAF6-p38MAPK signaling pathway in human middle ear epithelial cells. *BMC Infect. Dis.* 8, 87. <https://doi.org/10.1186/1471-2334-8-87>.
- Lee, H.Y., Kim, Y.I., Lee, J.W., Byun, J.Y., Park, M.S., Yeo, S.G., 2013. Decreased expression of TLR-9 and cytokines in the presence of bacteria in patients with otitis media with effusion. *Clin. Exp. Otorhinolaryngol.* 6, 195–200. <https://doi.org/10.3342/ceo.2013.6.4.195>.
- Leichtle, A., Hernandez, M., Pak, K., Yamasaki, K., Cheng, C.-F., Webster, N.J., Ryan, A.F., Wasserman, S.I., 2009. TLR4-mediated induction of TLR2 signaling is critical in the pathogenesis and resolution of otitis media. *Innate Immun.* 15, 205–215. <https://doi.org/10.1177/1753425909103170>.
- MacArthur, C.J., Pillers, D.-A.M., Pang, J., Kempton, J.B., Trune, D.R., 2011. Altered expression of middle and inner ear cytokines in mouse otitis media. *Laryngoscope* 121, 365–371. <https://doi.org/10.1002/lary.21349>.
- Mittal, R., Kodiyan, J., Gerring, R., Mathee, K., Li, J.-D., Grati, M., Liu, X.Z., 2014. Role of innate immunity in the pathogenesis of otitis media. *Int. J. Infect. Dis. Off. Publ. Int. Soc. Infect. Dis.* 29, 259–267. <https://doi.org/10.1016/j.ijid.2014.10.015>.
- Moon, S.-K., Lee, H.-Y., Pan, H., Takeshita, T., Park, R., Cha, K., Andalibi, A., Lim, D.J., 2006. Synergistic effect of interleukin 1 alpha on nontypeable *Haemophilus influenzae*-induced up-regulation of human beta-defensin 2 in middle ear epithelial cells. *BMC Infect. Dis.* 6, 12. <https://doi.org/10.1186/1471-2334-6-12>.
- Philpott, D.J., Sorbara, M.T., Robertson, S.J., Croitoru, K., Girardin, S.E., 2014. NOD proteins: regulators of inflammation in health and disease. *Nat. Rev. Immunol.* 14, 9–23. <https://doi.org/10.1038/nri3565>.
- Preciado, D., Goyal, S., Rahimi, M., Watson, A.M., Brown, K.J., Hathout, Y., Rose, M.C., 2010. MUC5B is the predominant mucin glycoprotein in chronic otitis media fluid. *Pediatr. Res.* 68, 231–236. <https://doi.org/10.1203/PDR.0b013e3181eb2ecc>.
- Shen, H., Yoshida, H., Yan, F., Li, W., Xu, F., Huang, H., Jono, H., Li, J.-D., 2008. Synergistic induction of MUC5AC mucin by nontypeable *Haemophilus influenzae* and *Streptococcus pneumoniae*. *Biochem. Biophys. Res. Commun.* 365, 795–800. <https://doi.org/10.1016/j.bbrc.2007.11.060>.
- Shimada, J., Moon, S.K., Lee, H.-Y., Takeshita, T., Pan, H., Woo, J.-I., Gellibolian, R., Yamanaka, N., Lim, D.J., 2008. Lysozyme M deficiency leads to an increased susceptibility to *Streptococcus pneumoniae*-induced otitis media. *BMC Infect. Dis.* 8, 134. <https://doi.org/10.1186/1471-2334-8-134>.
- Si, Y., Zhang, Z.G., Chen, S.J., Zheng, Y.Q., Chen, Y.B., Liu, Y., Jiang, H., Feng, L.Q., Huang, X., 2014. Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. *Hum. Immunol.* 75, 771–776. <https://doi.org/10.1016/j.humimm.2014.05.009>.
- Ubell, M.L., Kerschner, J.E., Wackym, P.A., Burrows, A., 2008. MUC2 expression in human middle ear epithelium of patients with otitis media. *Arch. Otolaryngol. Head Neck Surg.* 134, 39–44. <https://doi.org/10.1001/archoto.2007.10>.

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# Diseases of the circulatory system



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Diseases of the circulatory system include various pathologies of the heart and blood vessels. Together, these are diseases with the highest frequency in the general human population.

High blood cholesterol, high blood pressure, inflammation, tobacco use, and physical inactivity are well-known risk factors for cardiovascular diseases. While a genetic predisposition is unequivocally important in the pathogenesis of diseases of the circulatory system, environmental factors have a particularly high impact, and they can be prevented.

Atherosclerosis is the leading cause of death and disability. In atherosclerosis the plaque accumulates inside the artery and may narrow arteries resulting in stroke, coronary or peripheral artery disease, and other serious conditions.

High blood pressure is a principal risk factor for many serious conditions including stroke and heart attack. High blood pressure is the result of a complex interplay between lifestyle, hereditary, and environmental factors. Arterial hypertension is one of the most common diseases in the world. According to generalized data, about one-fourth of the human population suffers from arterial hypertension, and half of these people may not know about their condition.

Most cases of high blood pressure are classified as primary (essential) high blood pressure and a minority as secondary high blood pressure.

Pulmonary hypertension refers to increased blood pressure within the arteries of the lungs. It develops due to severe vasoconstriction of pulmonary arterioles and leads to clinical manifestations such as shortness of breath, chest pain, and fast heartbeat (i.e., palpitation or tachycardia).

Diseases of the heart muscle, known as cardiomyopathies, are well-known causes of heart failure. Hypertrophic cardiomyopathy is one of the most common inherited conditions worldwide. In hypertrophic cardiomyopathy a portion of the heart muscle (in the ventricles and intraventricular septum) becomes thickened and hypertrophic. That results in narrowing of the ventricles, increased heart muscle stiffness, and decreased ability of the heart to pump blood effectively. HCM is caused mostly by mutations in sarcomere proteins. Arrhythmogenic right ventricular dysplasia (ARVD) is a rare type of hereditary cardiomyopathy. It is characterized by hypokinetic areas predominantly the right ventricle with associated arrhythmias. ARVD is caused by mutations in genes encoding desmosomal and some other proteins. Nevertheless, details of the pathogenesis of ARVD are largely unknown. This is a fine illustration

of a hereditary disorder with known underlying genes, yet the molecular mechanisms leading to the progression of clinical manifestations are unclear.

Even though heart attacks and strokes are among the most common causes of death in older people, these disorders are not included in this chapter due to the complexity and variety of their pathophysiology above the cellular level.

## CHAPTER

## 8.1

## Atherosclerosis description

Atherosclerosis is a chronic disease of the arteries of the elastic and muscular-elastic type that arises as a result of lipid and protein metabolism and is accompanied by the deposition of cholesterol and some fractions of lipoproteins on the inner arterial walls. The deposits form atheromatous plaques. Subsequent proliferation of connective tissue (termed sclerosis) in them and calcification of the vessel wall lead to deformation and narrowing of the lumen until obstruction (occlusion of the vessel) occurs.

A thickening and loss of elasticity of the walls of arteries that occurs with formation of atherosclerotic plaques within the arterial intima. (*Medical Subject Headings, MeSH*, <https://meshb.nlm.nih.gov>).

Atherosclerosis is the significant manifestation in such groups of diseases as coronary artery disease (CAD or ischemic heart disease (IHD)) or peripheral artery diseases. Atherosclerosis is one of the leading causes of hypertension. The pathogenesis of atherosclerosis is called atherogenesis. Atherosclerotic lesions develop in several stages. During the initial stages, lipoproteins and leukocytes enter into the intima, the innermost layer of an artery. High levels of low-density lipoproteins (LDL) complexed with cholesterol constitute a significant risk factor for atherosclerosis. The subsequent death of artery cells and the formation and rearrangement of intercellular substances, as well as the calcification of vessels, occur. These processes are controlled by a variety of signals that are often multidirectional (Bokhari and Bokhari, 2017; Davis, 2005).

During the initial stages in the development of atherosclerosis, the passage of low-density lipoprotein (LDL) into the arterial wall leads to lipid deposition and visible plaque development. First, LDL becomes oxidized and then attracts monocytes and macrophages to the site of deposition to induce the formation of lipid-laden foam cells in the vessel wall.

**Pathway 1.** Low-density lipoprotein internalization and the accumulation of lipids in atherosclerosis (Fig. 1).

Oxidized LDL (oxLDL) in the vessel wall causes dysfunction of endothelium. Endothelial cells undergo apoptosis, release proteins and other molecules that degrade the extracellular matrix (ECM), attract inflammatory cells, cause blood clotting, and induce contraction of vascular smooth muscle cells (VSMCs). The rapid increase of intracellular ROS and reduction of NO are considered hallmarks of endothelial dysfunction.

**Pathway 2.** *Oxidized LDL causes endothelial dysfunction in atherosclerosis (Fig. 2).*

Endothelial dysfunction and lipid accumulation initiate a robust inflammatory response leading to macrophage and VSMC remodeling and arterial calcification.

**Pathway 3.** *Arterial wall inflammation and calcification in atherosclerosis (Fig. 3).*

## Key cellular contributors and processes

Calcification

Process

Calcification is an increase in the amount of calcium salts in a tissue.

Chemokines

Protein or gene

Chemokines are a family of a larger group of extracellular signaling molecules called cytokines. Chemokines are secreted low-molecular-weight proteins that can induce chemotaxis—the directed movement of a cell in response to a molecular stimulus.

Proinflammatory cytokines

Protein or gene

Cytokines are a broad list of small proteins released by immune cells that participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony-stimulating factor (GMCS-F) and others), secreted primarily by macrophages and T-helper cells, upregulate proinflammatory reactions.

## Pathway 1

### Low-density lipoprotein internalization and the accumulation of lipids in atherosclerosis (Fig. 1)

#### Incoming signals

Lipid metabolism and the passage of low-density lipoproteins (LDL) out of the arterial lumen and into the arterial wall are the basis for the development of atherosclerosis. LDL particles accumulate in the vessel wall and initiate the formation of atherosclerotic plaque formation. Macrophages internalize LDL, become enlarged and full of lipids, and then transform into foam cells. Parts of the foam cell and dying macrophages accumulate in vessel walls, thereby participating in atherosclerotic plaque formation.

Reduced levels of high-density lipoprotein (HDL) and cholesterol are known risk factors together with high levels of LDL. HDL is involved with the transport of excess cholesterol from macrophages and other cells, so it is vital for diminishing the accumulation of foam cells in atherosclerosis.

#### Outcome effects

Cholesterol-laden foam cells and macrophages lose their mobility, release proinflammatory cytokines and proteases (not shown) that together recruit more monocytes to the site, and launch the inflammation. In atherosclerotic lesions, oxidized LDLs are connected with insoluble crystals of ceroids/lipofuscin aggregates. Cholesterol crystals and lipofuscin induce lysosome destabilization and cell death. Cathepsin B and other products of dying cells activate the NLRP3 inflammasome cascade to perpetuate the chronic inflammation (not shown the pathway ([Shapiro and Fazio, 2017](#))).

#### Signaling

Lipids are absorbed from the intestine in the form of chylomicron. Chylomicron is a complex of lipids with the apolipoproteins A, C, E, and B (APOAs, APOCs, APOE, and APOB). It is cleaved by lipoprotein lipase (LPL) near the endothelial cell surface, providing free fatty acids and glycerol for utilization by the endothelial cells. LPL hydrolyzes the triglycerides. Enriched with cholesterol, chylomicron remnants are absorbed by adipose tissue and liver cells with the help of APOE. The APOC2 is an essential cofactor of LPL. Very LDL (VLDL) particles are assembled in the liver from triglycerides, cholesterol, and apolipoproteins. Triglyceride-enriched VLDL consists of APOB, APOCs, and APOE. LPL catalyzes the hydrolysis of triglycerides to form cholesterol-enriched LDL particles. LDL particles containing APOB can penetrate the endothelial barrier and deposit in the vessel

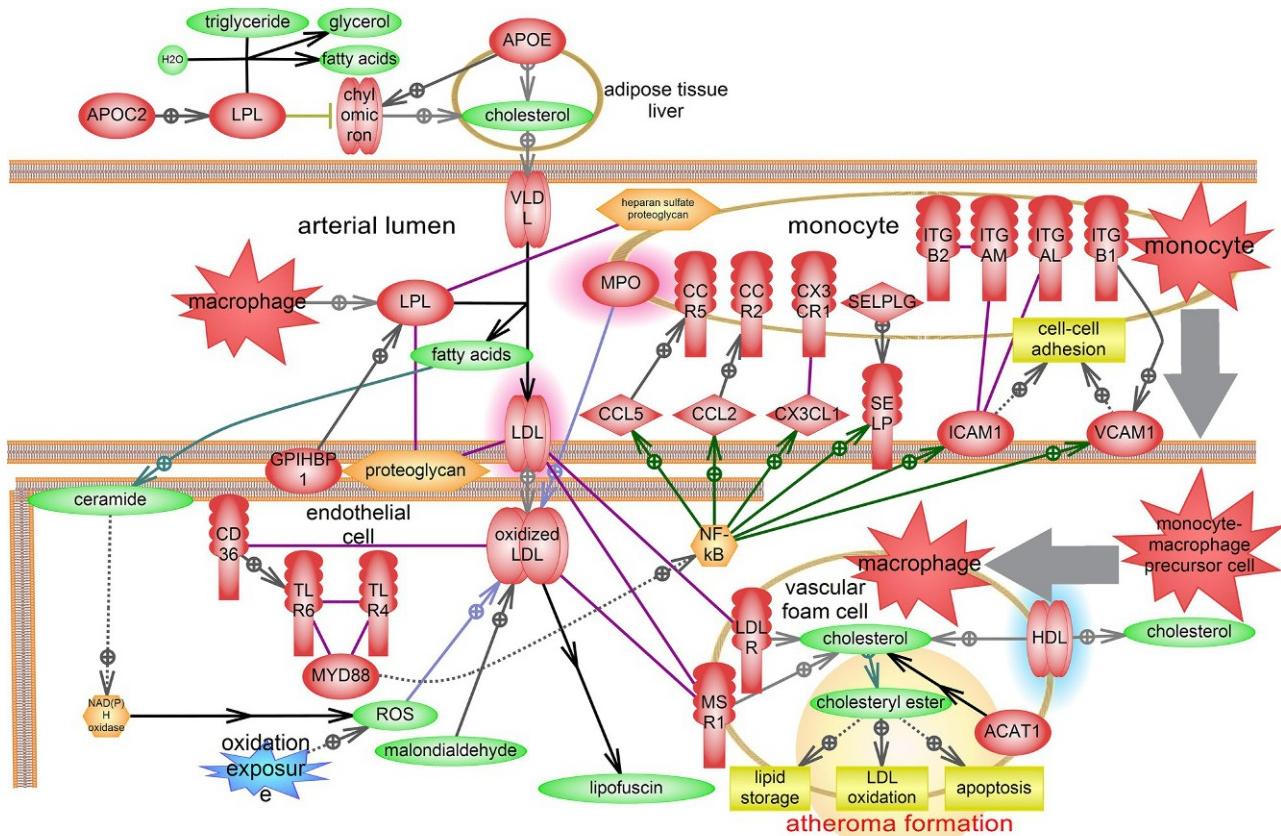
wall and bind to proteoglycans (Wu et al., 2017). In both pathways, LPL is a critical extracellular enzyme that enables tissues to import fatty acids from triacylglyceride-rich lipoproteins. The role of LPL in atherosclerosis is controversial. Probably, LPL circulating in plasma and secreted from adipose tissue and muscle cells binds to epithelial cells and exerts an antiatherogenic effect. When secreted from macrophages in the vessel wall, LPL has a proatherogenic effect. Moreover, LPL on the surface of the arterial endothelium, bound with proteoglycans or glycosylphosphatidylinositol anchored high-density lipoprotein-binding protein 1 (GPIHBP1), can also promote adhesion of monocytes to the endothelium (Li et al., 2014).

So, one of the first stages in the development of atherosclerosis is the passage of low-density lipoprotein (LDL) out of the arterial lumen and into the arterial wall. Once there the lipids in LDL are chemically modified and oxidized.

Oxidation of LDL can be stimulated by different oxidative agents including iron and other metals, hemoglobin, and mitochondrial ROS from vascular cells or phagocytes. One of the known oxidative agents is the lipid peroxidation product malondialdehyde (MDA) that can react with the LDL molecular complex, thus inducing lipid accumulation in macrophages. The presence of circulating MDA-LDL complexes was associated with the progression of atherosclerotic coronary artery disease (Martin-Ventura et al., 2017). Also, plasma levels of the protein myeloperoxidase (MPO) from activated monocytes, which produces oxidant HOCl molecules, are increased in atherosclerosis.

Oxidized LDL stimulates inflammatory signaling by neighboring endothelial cells (ECs), releasing chemokines and cytokines such as C-C motif chemokine ligand 5 (CCL5), C-X3-C motif chemokine ligand 1 (CX3CL1), and C-C motif chemokine ligand 2 (CCL2) in addition to intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) while recruiting monocytes into the arterial wall. Monocytes then differentiate into macrophages and internalize LDL with the help of the receptors low-density lipoprotein receptor (LDLR), macrophage scavenger receptor 1 (MSR1), and CD36. These macrophages continue to internalize LDL and oxidized LDL to become enlarged and full of lipid before transforming into foam cells (Chistiakov et al., 2017a; Davis, 2005; Luchtefeld et al., 2010; Woppard and Geissmann, 2010; Ylä-Herttula et al., 2011).

## II. Human disease pathways



**FIG. 1** Pathway 1: Low-density lipoprotein internalization and the accumulation of lipids in atherosclerosis.

## Pathway 2

### Oxidized LDL causes endothelial dysfunction in atherosclerosis (Fig. 2)

#### Incoming signals

Interaction of oxidized LDL (oxLDL) with vascular endothelial cells (ECs) plays an important role in the progression of atherosclerosis. oxLDL triggers endothelium dysfunction that is characterized by the decline of anticoagulants and vasodilating properties while increasing the release of proinflammatory mediators and ROS. A decrease in NO is considered a hallmark of endothelial dysfunction. Endothelial dysfunction disturbs the physiological protective regulatory balance that is a critical factor in atherosclerotic disease progression.

#### Outcome effects

Atherosclerosis-associated thickening of the arterial wall is usually connected with ECM remodeling, VSMC contraction, infiltration by inflammatory cells (monocytes, macrophages, T lymphocytes, etc.), and lipid deposition. The rapid increase of intracellular ROS results in vascular endothelium dysfunction leading to ECM degradation, the attraction of leukocytes and platelets, stimulation of blood clotting, and vasoconstriction. Also, ROS cause apoptosis of epithelial cells, thus enhancing the inflammatory reaction within the vessel. Reduction of NO causes constriction of vascular smooth muscle cells (VSMCs) and narrowing of the arterial lumen. Attracted to the arterial wall, macrophages contribute to the progression of atherosclerotic lesion and lipid plaque formation in the subendothelial region.

#### Signaling

Under oxidative stress, oxLDL in the vessel wall causes activation of oxidized low-density lipoprotein receptor 1 (OLR1) signaling in endothelial cells. In normal endothelium, OLR1 is expressed at low levels, whereas pathological states dramatically increase OLR1 expression. OxLDL, through OLR1, contributes to the induction of endothelial dysfunction by several mechanisms.

#### ***The first mechanism is NO and ROS production***

OLR1 activation triggers a membrane-bound NAD(P)H oxidase (NOX1-4) that results in the rapid increase of intracellular ROS. The exact mechanism of OLR1-dependent NAD(P)H oxidase activation is unknown.

By one hypothesis, OxLDL/OLR1 may induce ROS generation via the MMP14/RAC1-mediated axis. Also, oxLDL/OLR1 signaling inhibits NO production. OxLDL can prevent NO output by displacing nitric oxide synthase 3 (NOS3) from its caveolae membrane location by depleting caveolae of cholesterol (not shown). Also, oxLDL/OLR1 signaling may induce RHOA-dependent NOS3 protein downregulation. ARHGEF1 and ROCK2 may be involved in NOS3 protein downregulation. Furthermore, oxLDL activates endothelial arginase 2 (ARG2) that regulates NO production by competing with NOS3 for the common substrate L-arginine, through the dissociation of arginase from microtubules.

The upregulation of angiotensin I converting enzyme (ACE) expression in response to oxLDL/OLR1 leads to the conversion of angiotensin I to angiotensin II and may contribute to endothelial dysfunction. A derivative of angiotensinogen (AGT), angiotensin II, accumulates in the same vascular region as oxLDL. Angiotensin II is a potent stimulator of vascular ROS production via angiotensin II receptor type 1 (AGTR1) signaling. Angiotensin II also induces OLR1 expression and facilitates oxLDL uptake by endothelial cells (not shown).

### ***The decrease of intracellular NO results in increased levels of ROS***

ROS triggers activation of the NF- $\kappa$ B transcription factor and expression of several chemokines and adhesion molecules including CCL2, C-X-C motif chemokine ligand 2 (CXCL2), selectin E (SELE), and selectin P (SELP), which leads to the recruitment and adhesion of leukocytes to the endothelium.

### ***The second mechanism is the regulation of platelet adhesion and activation***

Endothelial OLR1 plays a chief role in platelet-endothelium interactions. OLR1 acts as an adhesion molecule for platelets. It binds to anionic phospholipids (phosphatidylserine) on the surface of activated platelets. oxLDL may partially inhibit the binding of platelets to OLR1. The adhesion of activated platelets to endothelial cells induces the release of EDN1 and triggers ROS generation by the endothelial cells.

OxLDL induces plasminogen activator inhibitor-1 (SERPINE1) upregulation in endothelial cells through OLR1 signaling ([Sangle et al., 2008](#)). SERPINE1 is an inhibitor of fibrinolytic enzymes.

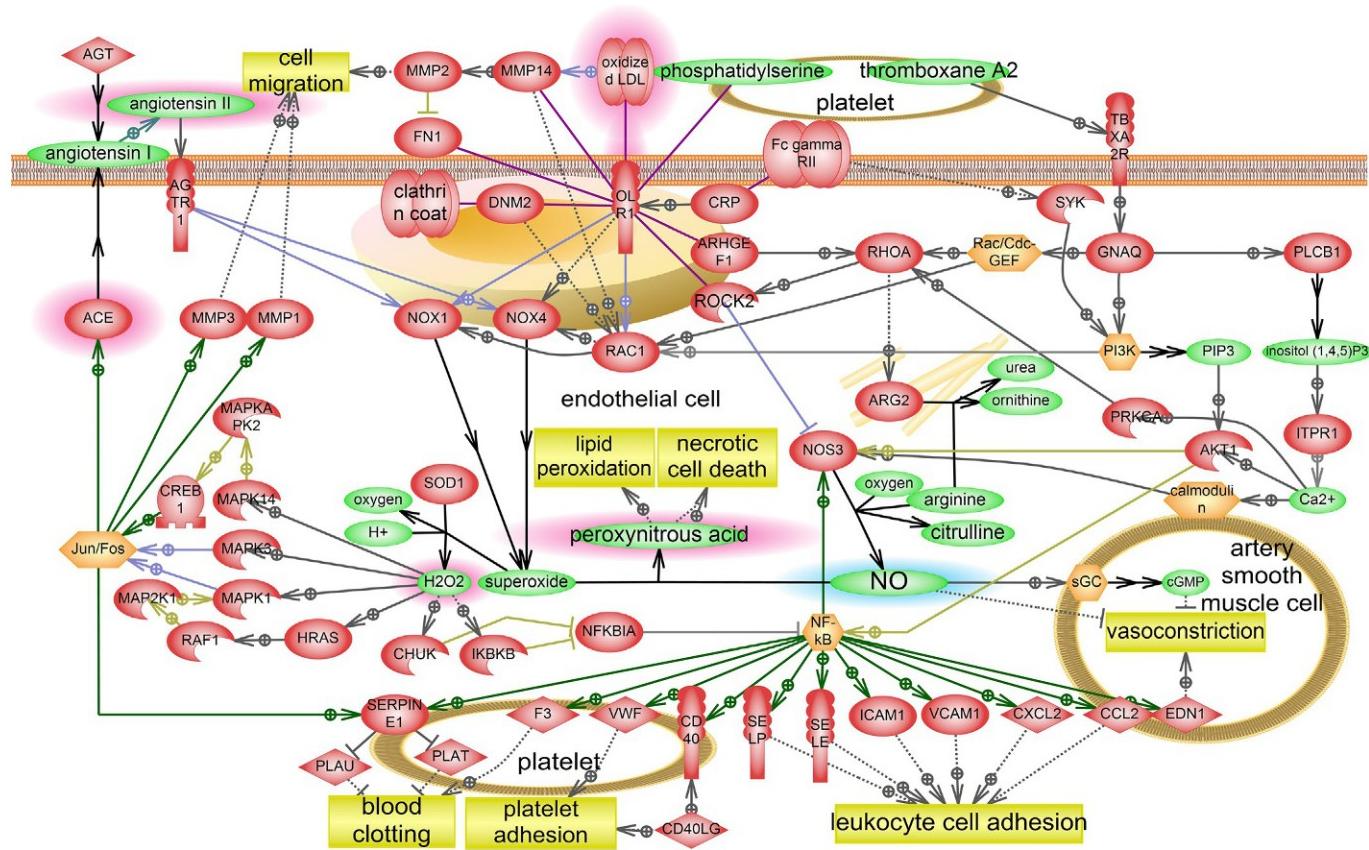
### ***The third mechanism is ECM remodeling***

OxLDL stimulates activation of matrix metalloproteinases (MMPs), which can cleave the extracellular matrix and thereby stimulate cell migration. OLR1 binds to MMP14, which is a cellular receptor and a potent matrix-degrading enzyme. In human endothelial cells the expression of MMP14 increases after treatment with oxLDL, possibly through an

EGFR-dependent mechanism (not shown). Also, oxLDL modulates the expression and activity of both MMP1 and MMP3 via OLR1 activation in human coronary artery endothelial cells.

C-reactive protein (CRP) and dynamin 2 (DNM2) along with some other molecules bind OLR1 and participate in oxLDL endocytosis. CRP, together with the Fc gamma-receptors II, has been shown to induce OLR1-related expression of ICAM1 and VCAM1 on human endothelial cells as well as RAC1 activation ([Galle et al., 2003](#); [Kattoor et al., 2017](#); [Mango et al., 2011](#); [Mattaliano et al., 2009](#); [Sakurai et al., 2004](#); [Sawamura et al., 2015](#)).

## II. Human disease pathways



**FIG. 2** Pathway 2: Oxidized LDL causes endothelial dysfunction in atherosclerosis.

## Pathway 3

### Arterial wall inflammation and calcification in atherosclerosis (Fig. 3)

#### Incoming signals

Atherosclerotic lesions frequently become calcified. Calcium deposits in coronary arteries are positive indicators of the development of atherosclerosis. There are known mutations in some genes implicated in phosphate/hydroxyapatite metabolism in some cases of idiopathic infantile arterial calcification. They include ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) and 5'-nucleotidase ecto (*NT5E*). Although intimal calcification associated with atherosclerosis is poorly understood, it is probably driven by the switch of the macrophage and vascular smooth cell phenotypes into osteoclast-like cells within the plaque site, or through apoptosis, or a general immune response to endothelial injury (Chistiakov et al., 2017b; Nicoll and Henein, 2017). Hydroxyapatite crystal accumulation in the ECM of the vessel wall is also believed to be involved in the observed calcification. Mönckeberg medial calcific sclerosis of the muscular middle layer of the vessel wall is different from atherosclerotic intimal calcification, and it too may be related to inflammation.

#### Outcome effects

Intima and plaque calcification in atherosclerosis are often detected in older patients. Calcifying vesicles can be observed microscopically in the collagen-rich matrix. Calcification escalates arterial stiffness and pulse wave velocity. In coronary arteries, calcification changes the physical properties of atherosclerotic plaques. Small calcium depositions increase the risk of atherosclerotic plaque rupture, while large calcification foci may even decrease this risk (Karwowski et al., 2012).

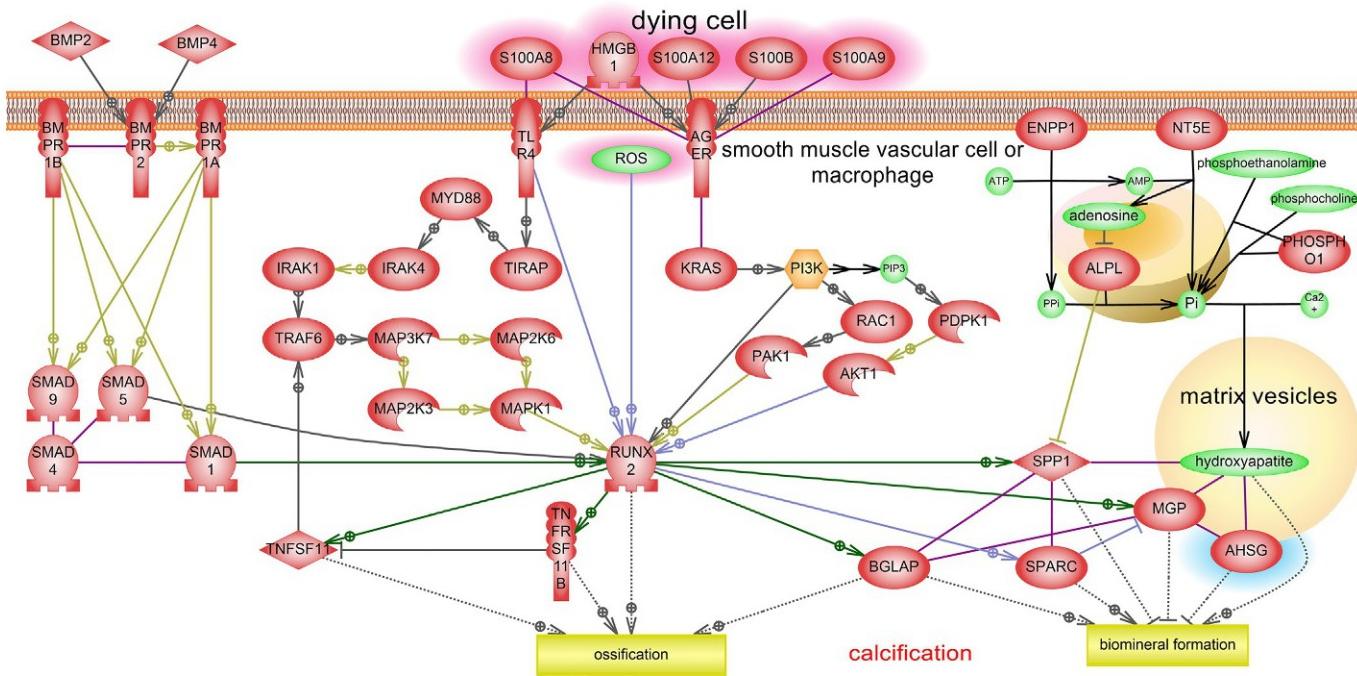
#### Signaling

Arterial wall calcification may share mechanisms with chondrocyte or osteoblast differentiation and bone mineralization. Proteins specific to bone development such as bone morphogenetic proteins 2 and 4 (BMP2/BMP4), secreted phosphoprotein 1 (SPP1), bone gamma-carboxyglutamate protein (BGLAP), secreted protein acidic and cysteine rich (SPARC), TNF receptor superfamily member 11b (TNFRSF11B), and TNF superfamily member 11 (TNFSF11) have been detected in calcified arteries (Otsuka et al., 2014).

Proteins associated with apoptosis including high mobility group box 1 (HMGB1) and S100 calcium binding proteins A8, A9, and

A12 (S100A8/S100A9/S100A12) mediate the TLR4 and advanced glycosylation end product-specific receptor (AGER) receptor cascades to initiate a proinflammatory response in macrophage and smooth vascular cells and probably contribute to increased cell calcification. AGER and TLR4 activate several signal transduction cascades including the family of MAPKs, PI3K, and some transcription factors such as NF- $\kappa$ B and JUN/FOS, which, in turn, leads to increased production of proinflammatory factors such as IL-6, CCL2, ICAM1, VCAM1, SERPINE1, and EDN1 (not shown). The precise mechanisms of intimal calcification remain unclear. The pathway shows an example of a hypothetical signaling cascade that could be responsible for cell calcification. Runt-related transcription factor 2 (RUNX2) is a well-known transcriptional factor involved in osteoclast-like differentiation and various signals involved in RUNX2 activation including the BMP or TLR2/TLR4 pathways. Decreased levels of protein inhibitors of apatite such as alpha-2-HS glycoprotein (AHSG) or secreted phosphoprotein 1 (SPP1) were associated with augmented vascular calcification in hemodialysis patients (Averill et al., 2012; Chistiakov et al., 2017b; Luchtefeld et al., 2010; Nitschke et al., 2012; Otsuka et al., 2014; St. Hilaire et al., 2011).

## II. Human disease pathways



**FIG. 3** Pathway 3: Arterial wall inflammation and calcification in atherosclerosis.

## References

- ICD-10: disease code I70. Diseases of the circulatory system (I00-I99). (ICD-10, <https://icdlist.com>). ICD-11: disease code BD40.
- Averill, M.M., Kerkhoff, C., Bornfeldt, K.E., 2012. S100A8 and S100A9 in cardiovascular biology and disease. *Arterioscler. Thromb. Vasc. Biol.* 32, 223–229. <https://doi.org/10.1161/ATVBAHA.111.236927>.
- Bokhari, M.R., Bokhari, S.R.A., 2017. Renal artery stenosis. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Chistiakov, D.A., Melnichenko, A.A., Orekhov, A.N., Bobryshev, Y.V., 2017a. How do macrophages sense modified low-density lipoproteins? *Int. J. Cardiol.* 230, 232–240. <https://doi.org/10.1016/j.ijcard.2016.12.164>.
- Chistiakov, D.A., Myasoedova, V.A., Melnichenko, A.A., Grechko, A.V., Orekhov, A.N., 2017b. Calcifying matrix vesicles and atherosclerosis. *Biomed. Res. Int.* 2017, 7463590. <https://doi.org/10.1155/2017/7463590>.
- Davis, N.E., 2005. Atherosclerosis—an inflammatory process. *J. Insur. Med.* 37, 72–75.
- Galle, J., Quaschning, T., Seibold, S., Wanner, C., 2003. Endothelial dysfunction and inflammation: what is the link? *Kidney Int. Suppl.*, S45–S49. <https://doi.org/10.1046/j.1523-1755.63.s84.12.x>.
- Karwowski, W., Naumnik, B., Szczepański, M., Myśliwiec, M., 2012. The mechanism of vascular calcification—a systematic review. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* 18, RA1–11.
- Kattoor, A.J., Pothineni, N.V.K., Palagiri, D., Mehta, J.L., 2017. Oxidative stress in atherosclerosis. *Curr. Atheroscler. Rep.* 19, 42. <https://doi.org/10.1007/s11883-017-0678-6>.
- Li, Y., He, P.-P., Zhang, D.-W., Zheng, X.-L., Cayabyab, F.S., Yin, W.-D., Tang, C.-K., 2014. Lipoprotein lipase: from gene to atherosclerosis. *Atherosclerosis* 237, 597–608. <https://doi.org/10.1016/j.atherosclerosis.2014.10.016>.
- Luchtefeld, M., Grothusen, C., Gagalick, A., Jagavelu, K., Schuett, H., Tietge, U.J.F., Pabst, O., Grote, K., Drexler, H., Forster, R., Schieffer, B., 2010. Chemokine receptor 7 knockout attenuates atherosclerotic plaque development. *Circulation* 122, 1621–1628. <https://doi.org/10.1161/CIRCULATIONAHA.110.956730>.
- Mango, R., Predazzi, I.M., Romeo, F., Novelli, G., 2011. LOX-1/LOXIN: the yin/yang of atherosclerosis. *Cardiovasc. Drugs Ther.* 25, 489–494. <https://doi.org/10.1007/s10557-011-6333-5>.
- Martin-Ventura, J.L., Rodrigues-Diez, R., Martinez-Lopez, D., Salaises, M., Blanco-Colio, L.M., Briones, A.M., 2017. Oxidative stress in human atherothrombosis: sources, markers and therapeutic targets. *Int. J. Mol. Sci.* 18. <https://doi.org/10.3390/ijms18112315>.
- Mattaliano, M.D., Huard, C., Cao, W., Hill, A.A., Zhong, W., Martinez, R.V., Harnish, D.C., Paulsen, J.E., Shih, H.H., 2009. LOX-1-dependent transcriptional regulation in response to oxidized LDL treatment of human aortic endothelial cells. *Am. J. Physiol. Cell Physiol.* 296, C1329–C1337. <https://doi.org/10.1152/ajpcell.00513.2008>.
- Nicoll, R., Henein, M., 2017. Arterial calcification: a new perspective? *Int. J. Cardiol.* 228, 11–22. <https://doi.org/10.1016/j.ijcard.2016.11.099>.
- Nitschke, Y., Baujat, G., Botschen, U., Wittkampf, T., du Moulin, M., Stella, J., Le Merrer, M., Guest, G., Lambot, K., Tazaroute-Pinturier, M.-F., Chassaing, N., Roche, O., Feenstra, I., Loechner, K., Deshpande, C., Garber, S.J., Chikarmane, R., Steinmann, B., Shahinyan, T., Martorell, L., Davies, J., Smith, W.E., Kahler, S.G., McCulloch, M., Wraige, E., Loidi, L., Höhne, W., Martin, L., Hadj-Rabia, S., Terkeltaub, R., Rutsch, F., 2012. Generalized arterial calcification of infancy and pseudoxanthoma elasticum can be caused by mutations in either ENPP1 or ABCC6. *Am. J. Hum. Genet.* 90, 25–39. <https://doi.org/10.1016/j.ajhg.2011.11.020>.
- Otsuka, F., Sakakura, K., Yahagi, K., Joner, M., Virmani, R., 2014. Has our understanding of calcification in human coronary atherosclerosis progressed? *Arterioscler. Thromb. Vasc. Biol.* 34, 724–736. <https://doi.org/10.1161/ATVBAHA.113.302642>.

- Sakurai, K., Cominacini, L., Garbin, U., Fratta Pasini, A., Sasaki, N., Takuwa, Y., Masaki, T., Sawamura, T., 2004. Induction of endothelin-1 production in endothelial cells via co-operative action between CD40 and lectin-like oxidized LDL receptor (LOX-1). *J. Cardiovasc. Pharmacol.* 44 (Suppl. 1), S173–S180.
- Sangle, G.V., Zhao, R., Shen, G.X., 2008. Transmembrane signaling pathway mediates oxidized low-density lipoprotein-induced expression of plasminogen activator inhibitor-1 in vascular endothelial cells. *Am. J. Physiol. Endocrinol. Metab.* 295, E1243–E1254. <https://doi.org/10.1152/ajpendo.90415.2008>.
- Sawamura, T., Wakabayashi, I., Okamura, T., 2015. LOX-1 in atherosclerotic disease. *Clin. Chim. Acta Int. J. Clin. Chem.* 440, 157–163. <https://doi.org/10.1016/j.cca.2014.11.016>.
- Shapiro, M.D., Fazio, S., 2017. Apolipoprotein B-containing lipoproteins and atherosclerotic cardiovascular disease. *F1000Research* 6, 134. <https://doi.org/10.12688/f1000research.9845.1>.
- St. Hilaire, C., Ziegler, S.G., Markello, T.C., Brusco, A., Groden, C., Gill, F., Carlson-Donohoe, H., Lederman, R.J., Chen, M.Y., Yang, D., Siegenthaler, M.P., Arduino, C., Mancini, C., Freudenthal, B., Stanescu, H.C., Zdebik, A.A., Chaganti, R.K., Nussbaum, R.L., Kleta, R., Gahl, W.A., Boehm, M., 2011. *NT5E* mutations and arterial calcifications. *N. Engl. J. Med.* 364, 432–442. <https://doi.org/10.1056/NEJMoa0912923>.
- Woollard, K.J., Geissmann, F., 2010. Monocytes in atherosclerosis: subsets and functions. *Nat. Rev. Cardiol.* 7, 77–86. <https://doi.org/10.1038/nrcardio.2009.228>.
- Wu, M.-Y., Li, C.-J., Hou, M.-F., Chu, P.-Y., 2017. New insights into the role of inflammation in the pathogenesis of atherosclerosis. *Int. J. Mol. Sci.* 18. <https://doi.org/10.3390/ijms18102034>.
- Ylä-Herttula, S., Bentzon, J.F., Daemen, M., Falk, E., Garcia-Garcia, H.M., Herrmann, J., Hoefer, I., Jukema, J.W., Kramps, R., Kwak, B.R., Marx, N., Naruszewicz, M., Newby, A., Pasterkamp, G., Serruys, P.W.J.C., Waltenberger, J., Weber, C., Tokgözoglu, L., 2011. Stabilisation of atherosclerotic plaques: position paper of the European Society of Cardiology (ESC) Working Group on atherosclerosis and vascular biology. *Thromb. Haemost.* 106, 1–19. <https://doi.org/10.1160/TH10-12-0784>.

## CHAPTER

## 8.2

## Hypertension

Hypertension (arterial hypertension or high blood pressure) is a chronic increase in blood pressure. Hypertension is the most significant risk factor for a wide range of cardiovascular diseases such as stroke, congestive heart failure, peripheral vascular disease, and renal disease.

Normal blood pressure (BP) in adults can be defined as systolic BP <120 mm Hg and diastolic BP <80 mm Hg. Worldwide, it is estimated that 41% of people ages 35 to 70 years have hypertension, and only 46.5% of them are aware of it. (*Ferri and Ferri, 2018*).

Hypertension is subdivided into essential hypertension (primary hypertension or idiopathic hypertension) and secondary hypertension. Ninety to ninety-five percent of all cases of hypertension are classified as essential hypertension. Essential hypertension is a multifactorial disease that simultaneously depends on both hereditary and lifestyle elements including diet with excess salt, obesity, lack of exercise, alcohol, smoking, and stress. Secondary hypertension is defined as high blood pressure triggered by some recognizable causes such as chronic kidney and endocrine diseases. The diagnosis of hypertension is complicated by the fact that it is typically not accompanied by symptoms for a long time.

In general, hypertension is caused by the interplay of several factors such as (a) activation of the renin-angiotensin-aldosterone system and sympathetic nervous system; (b) increased renal tubular reabsorption of salt and water; (c) decreased synthesis of nitric oxide and natriuretic factors; and (d) increased production of endothelin, reactive oxygen species, and inflammatory cytokines (*Currie and Delles, 2016*). Hypertension with known inheritance patterns can be divided into the group of monogenic familial syndromes with malignant progression and other genetic predispositions, which underlie individual high blood pressure progression. Besides, genome-wide association studies (GWAS) have identified numerous variations in many genes, including *AGT*, *AGTR1*, *CYP11B1/CYP11B2*, *SCNN1A/SCNN1B/SCNN1G*, *SLC12A1*, *WNK1/WNK4*, *NR3C2*, *KCNJ15*, *CLCNKA1*, *CLCNKB*, *CASR*, and *CACNA1D*, implicated in high blood

pressure pathogenesis in diverse populations and groups of different age or gender (Botzer et al., 2018; Burrello et al., 2017; Ehret and Caulfield, 2013) (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Hypertension develops gradually and consistently. It includes several stages and depends on complex interactions of genetic, neurohumoral and psychoemotional factors.

An imbalance in the number of factors regulating cardiovascular system activity may cause the observed increase in arterial pressure. For example, higher levels of aldosterone change salt and water homeostasis that in turn leads to hypertension:

**Pathway 1.** *Rise in aldosterone synthesis in adrenal zona glomerulosa cells (Fig. 4).*

**Pathway 2.** *The role of aldosterone in the regulation of  $\text{Na}^+/\text{Cl}^-/\text{K}^+$  homeostasis (Fig. 5).*

High levels of aldosterone and other mediators cause constriction of smooth muscle cells that leads to a narrowing of the lumen of blood vessels and lead to high blood pressure.

**Pathway 3.** *Dysfunction of smooth muscle cells in hypertension (Fig. 6).*

**Pathway 4.** *Endothelial cells repress vasoconstriction in hypertension (Fig. 7).*

Increased blood pressure leads to an increase in cardiovascular system stress and damage. Arterial hypertension significantly increases the risk of other cardiovascular complications.

## Key cellular contributors and processes

Adrenal zona glomerulosa

Anatomic structure

Adrenal zona glomerulosa is the outermost layer of adrenal cortex that produces the major mineralocorticoid hormone, aldosterone.

Aldosterone

Protein or gene

Aldosterone is a key mineralocorticoid hormone that has a crucial role in maintaining the electrolyte and water balance in the body and, thus, is important for blood pressure regulation.

Blood pressure

Process

Blood pressure is the pressure of circulating blood on the walls of blood vessels.

Diastolic blood pressure

Process

Diastolic pressure is the minimal pressure of circulating blood on the arterial walls during heart relaxation.

Endothelial cells

Cell

Endothelial cells (ECs) are single-layered cells that line the inside of blood and lymph vessels and mediate the selective movement of substances and cells between the bloodstream and surrounding cells.

Homeostasis

Process

Homeostasis is self-regulation, the ability of a system to maintain stable equilibrium and constancy of its internal state through coordinated reactions.

Smooth muscle cells of blood vessels

Cell

Smooth muscle cells (SMC) of blood vessels are nonstriated muscle cells found in the middle layer of the vascular wall.

Systolic blood pressure

Process

Systolic blood pressure is the pressure of circulating blood on the arterial walls at the moment of heart muscle contraction.

## Pathway 1

### Rise in aldosterone synthesis in adrenal zona glomerulosa cells (Fig. 4)

#### Incoming signals

Aldosterone plays an essential role in salt and water homeostasis and correspondingly in blood pressure regulation. Chronic activation of the aldosterone secretion system (specifically the renin-angiotensin-aldosterone system, RAAS) is a major contributing factor to the pathogenesis and progression of arterial hypertension.

Aldosterone is synthesized by the adrenal zona glomerulosa cells in response angiotensin II (Ang II) and adrenocorticotropic hormone (ACTH) stimulation. These stimuli can have a chronic and acute influence on aldosterone production.

High renin and Ang II levels are two risk factors of hypertension, at least in some hypertension patients, especially those with familial syndromes. *CYP11B2* gene polymorphisms were associated with an increased risk of hypertension. Moreover the chimeric product of the *CYB11B2/CYP11B1* genes was identified in patients with high blood pressure in primary hyperaldosteronism (in particular familial hyperaldosteronism type I) (OMIM: 103900). A fused protein consisting of exons 1–4 of *CYP11B1* and exons 5–9 of *CYP11B2* leads to excessive aldosterone production.

Endothelin 1 (EDN1) also acts as an independent stimulator of aldosterone secretion in adrenal zona glomerulosa cells. EDN1 is secreted by both endothelial cells and adrenal zona glomerulosa cells in response to specific stimuli such as hypoxia.

#### Outcome effects

High aldosterone levels disturb salt and water homeostasis in the kidney epithelium and provoke the progression of arterial hypertension (see Pathways 2 and 3).

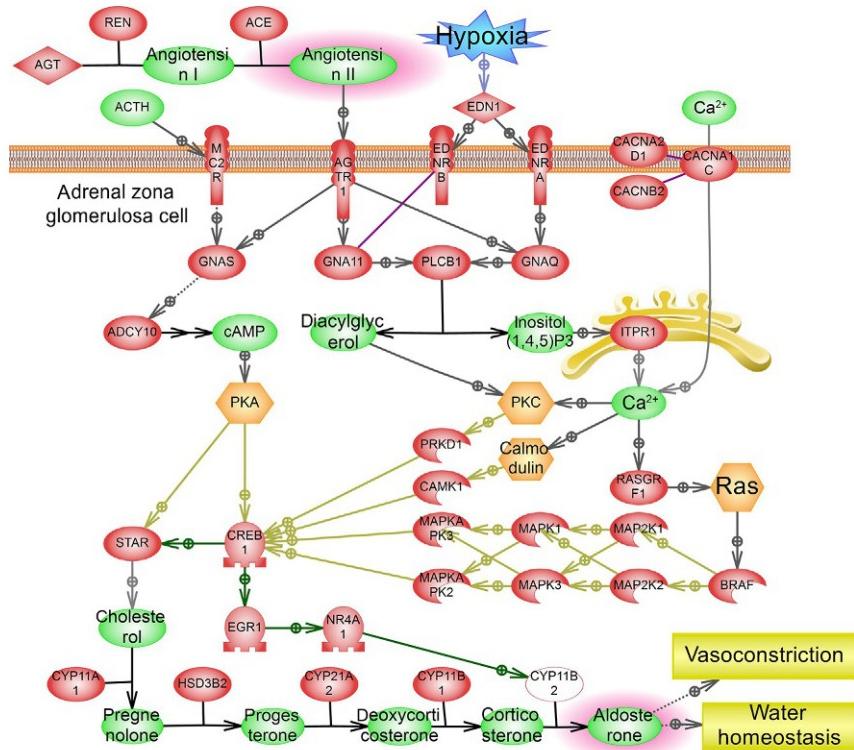
#### Signaling

The classic RAAS is a cascade composed of several different components. The juxtaglomerular apparatus in the kidney secretes renin (REN) in response to decreased intrarenal pressure and low  $\text{Na}^+$  and  $\text{K}^+$  levels. REN cleaves the inactive angiotensinogen (AGT) released from the liver converting it into angiotensin I. Angiotensin I is then converted into active angiotensin II by angiotensin-converting enzyme (ACE). Additional RAAS components that participate in intracellular Ang II modifications were discovered (not shown).

Angiotensin II and EDN1 bind to their receptors (AGTR1 and ENDRA and ENDRB, respectively) on adrenal zona glomerulosa cell membrane. The activity of their receptors is mediated by the G proteins GNA11 and GNAQ. G proteins, in turn, activate phospholipase C beta (PLCB1), an enzyme that mediates diacylglycerol and inositol 1,4,5-trisphosphate production. Inositol 1,4,5-trisphosphate binds to its receptor, ITPR1, on the endoplasmic reticulum and results in increased cytosolic  $\text{Ca}^{2+}$  concentrations.  $\text{Ca}^{2+}$  in turn induces the MAPK cascade, protein kinase C (PKC), and calmodulin-dependent kinase 1 (CAMK1), leading to activation of the transcription factor CREB1. CREB1 upregulates steroidogenic acute regulatory protein (STAR) transcription leading to increased synthesis of the encoded protein that mediates the transport of cholesterol into the mitochondria.

Cholesterol is a primary precursor for aldosterone synthesis. Cytochrome P450 11A1 (CYP11A1) converts cholesterol to pregnenolone, and hydroxy-delta-5-steroid dehydrogenase (HSD3B2) catalyzes the oxidative conversion pregnenolone to progesterone. Progesterone is then hydroxylated to desoxycorticosterone by cytochrome P450 21A2 (CYP21A2). Cytochrome P450 11B1 (CYP11B1) and cytochrome P450 11B2 (CYP11B2 also known as aldosterone synthase) convert desoxycorticosterone to corticosterone and then corticosterone to aldosterone.

MC2R is the receptor for ACTH, transmitting the signal to adenylate cyclase 10 (ADCY10) within the cell via the regulatory protein GNAS. ADCY10 produces the second messenger cAMP, thereby stimulating the activity of PKA that phosphorylates and activates CREB1 and STAR (Baudrand and Vaidya, 2018; Hattangady et al., 2012; Shah et al., 2006; Te Riet et al., 2015).



**FIG. 4** Pathway 1: Rise in aldosterone synthesis in adrenal zona glomerulosa cells.

## Pathway 2

### The role of aldosterone in the regulation of $\text{Na}^+/\text{Cl}^-/\text{K}^+$ homeostasis (Fig. 5)

#### Incoming signals

Aldosterone regulates the activity of ion channels that control electrolyte transport across epithelia in the nephron. Those channels are responsible for  $\text{Na}^+$  and  $\text{Cl}^-$  reabsorption into the blood and  $\text{K}^+$  excretion in the distal renal tubules.

Mutations in the *WNK1* and *WNK4 genes* and excessively high aldosterone level are the main reasons for increased  $\text{Na}^+$  ion levels in the blood leading to increased blood volume and high blood pressure. The WNK lysine-deficient protein kinases (*WNK1* and *WNK4*) are regulators of electrolyte homeostasis. They are associated with familial hyperkalemic hypertension (or familial pseudohypoaldosteronism type II). Deletions in the *WNK1 gene* lead to increased enzymatic activity and activation of the redundant *WNK1* long-isoform (L-WNK1). Increased levels of the *WNK4* protein are associated with familial hyperkalemic hypertension variants in the *WNK4 gene*. Enhancing L-WNK1 and *WNK4* protein activity leads to abnormally high  $\text{Na}^+$  reabsorption and blocking of  $\text{K}^+$  excretion, resulting in hypertension. Polymorphisms in the *WNK1* and *WNK4* genes are also associated with an increased risk of nonsyndromal hypertension.

#### Outcome effects

Excessive  $\text{Na}^+$  levels in plasma are the reason for increased blood volume and high blood pressure. So the maintenance of  $\text{Na}^+/\text{Cl}^-/\text{K}^+$  homeostasis by the kidney system is necessary for the maintenance of normal concentrations of ions and healthy blood pressure regulation.

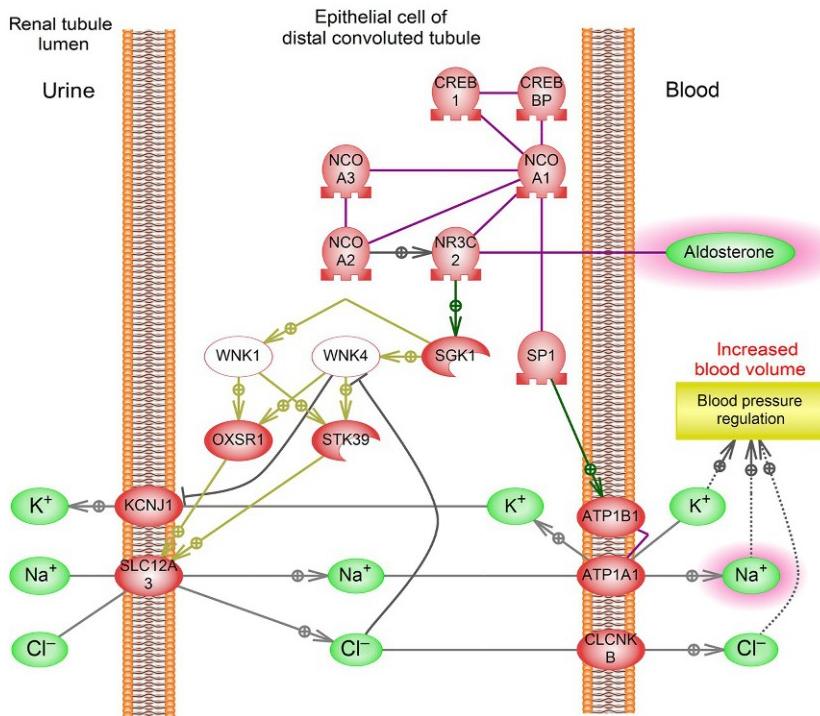
#### Signaling

Aldosterone in kidney epithelial cells of the distal convoluted tubules binds with the nuclear mineralocorticoid receptor NR3C2 to activate production of the serum/glucocorticoid-regulated kinase 1 (SGK1).

SGK1 phosphorylates *WNK1* and *WNK4* that in turn controls the transport of  $\text{Na}^+$  and  $\text{Cl}^-$  ions by the membrane cotransporter SLC12A3 via the serine/threonine protein kinase OXSR1 and serine/threonine kinase 39 (STK39). *WNK4* inhibits the potassium channel KCNJ1 and reduces the  $\text{K}^+$  excretion from cells into urine. SLC12A3 mediates  $\text{Na}^+$  and  $\text{Cl}^-$  reabsorption from urine to the cells. Uninhibited KCNJ1 transfers  $\text{K}^+$  from renal cells into urine.

The precise mechanisms of WNK activity regulation are still unclear. But it was shown that  $\text{Cl}^-$  ions bind to WNK4 and inhibit its activity.

In addition to SLC12A3 and KCNJ1, the  $\text{Na}^+/\text{K}^+$  ATPase channel (ATP1A1 and ATP1B1) and chloride channel CLCNKB also control  $\text{Na}^+/\text{K}^+/\text{Cl}^-$  ion transport in the distal renal tubules of nephrons. CLCNKB transports  $\text{Cl}^-$  ions from cells into the blood. ATP1A1 and ATP1B1 (a heterodimeric complex consisting of an alpha (ATP1A1) and beta (ATP1B1) subunit) transports  $\text{Na}^+$  ions from cells into the blood and  $\text{K}^+$  ions from blood into renal cells. Transcription of the *ATP1B1* gene in the kidney is modulated by aldosterone via the specificity transcription factor SP1 ([Dbouk et al., 2016](#); [Delles et al., 2010](#); [Hamm and Hering-Smith, 2010](#); [Murthy et al., 2017](#)).



**FIG. 5** Pathway 2: The role of aldosterone in the regulation of  $\text{Na}^+/\text{Cl}^-/\text{K}^+$  homeostasis.

## Pathway 3

### Dysfunction of smooth muscle cells in hypertension (Fig. 6)

#### Incoming signals

Strengthened vasoconstriction is believed to be an early component during the development of arterial hypertension and is associated with smooth muscle cell (SMC) dysfunction. Redundant levels of angiotensin II, norepinephrine (noradrenaline), vasopressin (AVP) and endothelin 1 (EDN1) promote smooth muscle cell proliferation and cellular growth and induce muscular contraction.

#### Outcome effects

Normal physiological SMC activity regulates arterial wall tone and vascular lumen size. Excessive vascular contraction, SMC proliferation, and cellular growth lead to arterial wall thickening, vessel lumen narrowing, and eventually high blood pressure and cardiovascular system obsolescence.

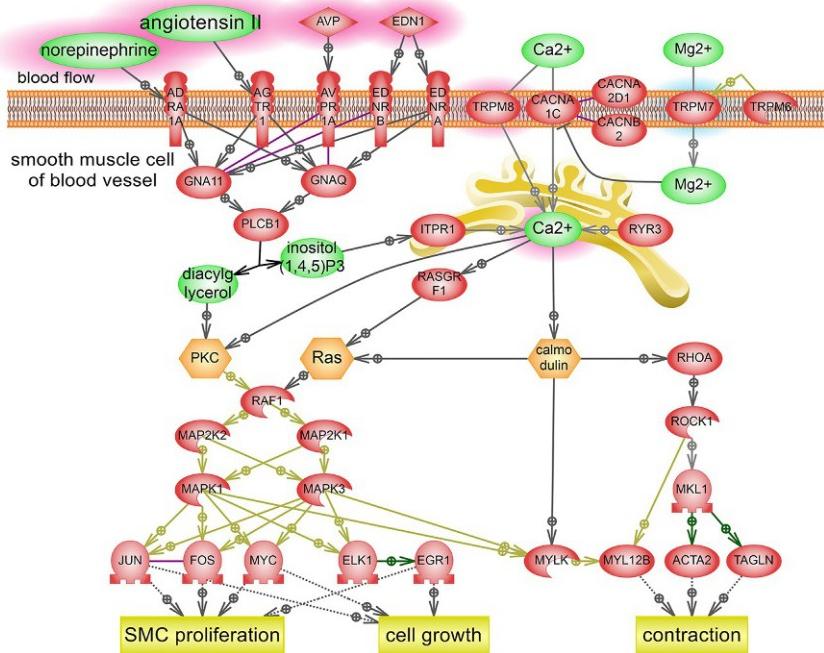
#### Signaling

Angiotensin II, norepinephrine, AVP, and EDN1 receptors are regulated by the G proteins GNA11 and GNAQ, which in turn activate the phospholipase C beta (PLCB1) and phosphatidylinositol-calcium second messenger system. PLCB1 mediates the production of both diacylglycerol and inositol 1,4,5-trisphosphate. Diacylglycerol activates protein kinase C (PKC). Inositol 1,4,5-trisphosphate binds to its receptor ITPR1 on the endoplasmic reticulum and promotes  $\text{Ca}^{2+}$  release into the cytoplasm.  $\text{Ca}^{2+}$  release is also induced via the ryanodine receptor (RyR3) in the sarcoplasmic reticulum.

Smooth muscle cells may absorb extracellular  $\text{Ca}^{2+}$  through voltage-dependent calcium channels (CACNA1C, CACNA2D1, and CACNB2). Nonspecific transient cation channels (TRPMs) also play an essential role in  $\text{Ca}^{2+}$  uptake. TRPM6 interacts with TRPM7 to form a heterotetrameric channel important for  $\text{Mg}^{2+}$  homeostasis. Typically,  $\text{Mg}^{2+}$  blocks CACNA1C channels, reducing  $\text{Ca}^{2+}$  entry into smooth muscle cells. Low levels of TRPM7 expression were found in patients with hypertension and can cause a reduced intracellular  $\text{Mg}^{2+}$  uptake.

$\text{Ca}^{2+}$  is a highly versatile intracellular signal that induces the MAPK cascade through calmodulin, PKC, and the Ras-specific exchange factor RASGRF1 activation. MAPKs activate the transcription factors JUN, FOS, MYC, ELK1, and EGR1, which are involved in SMC proliferation

and growth. MAPK1 and MAPK3 activates myosin light chain kinase (MYLK) that phosphorylates the myosin regulator MYL12B to facilitate the contractile activity. Calmodulin stimulates activation of transforming protein RHOA that, through protein kinase ROCK1 and transcription factor MKL1, increases synthesis of alpha-actin 2 (ACTA2), and transgelin (TAGLN) is involved in smooth muscle cell contraction (Kohan et al., 2011; Loirand and Pacaud, 2014; Rush and Aultman, 2008; Yogi et al., 2011).



**FIG. 6** Pathway 3: Dysfunction of smooth muscle cells in hypertension.

## Pathway 4

### Endothelial cells repress vasoconstriction in hypertension (Fig. 7)

#### Incoming signals

The vascular endothelium plays a crucial role in the regulation of vasoconstriction via the synthesis and release of vasodilator substances such as nitric oxide (NO) and prostaglandins. The innermost layer of the vascular arterial wall consists of a monolayer of endothelial cells and connective tissue. Vasodilators can pass through the connective tissue between the endothelial cells and can be absorbed by the smooth muscle cell (SMC) layer underlying the endothelium. NO production can be stimulated by bradykinin, histamine, and other substances. A bradykinin deficiency and a lack of constitutional activation of nitric oxide synthase (NOS3) lead to a shortage of NO and prostaglandin and therefore impaired vasodilation.

#### Outcome effects

Endothelium is a monolayer tissue that regulates interactions between the arterial wall and many cells and factors coming from circulating blood such as immune cells, hormones, and mediators of cellular function. Disturbance of endothelium-dependent vasodilation leads to endothelial dysfunction and disruption of the targeted action of vasoactive molecules on the vascular muscle tone.

#### Signaling

Bradykinin is formed by the kinin-kallikrein system in the blood. Bradykinin is produced by plasma kallikrein (KLKB1) from kininogen (KNG1). Bradykinin and histamine both act through their respective receptors (BDKRB2 and HRH1) whose activities are mediated by the G protein GNAQ. GNAQ activates phospholipase C beta (PLCB1) that leads to diacylglycerol and inositol 1,4,5-trisphosphate production. Inositol 1,4,5-trisphosphate binds with its receptor ITPR1 on the endoplasmic reticulum to cause  $\text{Ca}^{2+}$  ion release into the cytoplasm.  $\text{Ca}^{2+}$  ions in turn activate PI3K via calmodulin, leading to PIP3 synthesis. Also, GNAQ binds to phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CA/PIK3R1) to activate AKT1 via PIP3 and the 3-phosphoinositide-dependent protein kinase-1 (PDPK1). AKT1 phosphorylates NOS3, producing the main vasodilator NO from its precursor extracellular L-arginine. NO activates prostaglandin synthases (PTGS1 and PTGS2), which convert arachidonic acid to prostaglandin H2 (PGH2). The prostaglandin E synthases (PTGES

and PTGES2) catalyze the isomerization of PGH<sub>2</sub> into prostaglandin E2. Under healthy conditions, endothelium-derived NO activates soluble guanylate cyclase (sGC) in SMC. sGC produces cGMP that prevents vasoconstriction through myosin light chain kinase (MLK) inhibition caused by the cGMP-dependent protein kinase 1 (PRKG1). Endothelium-derived prostaglandin E2 binds with the prostaglandin E receptor (PTGER4) on SMC membranes and activates the adenylate cyclase (ADCY) through the G protein GNAS to produce cAMP and mediate PKA activity. PKA-related inhibition of MLK leads to vasodilation ([Konukoglu and Uzun, 2017](#); [Versari et al., 2009](#)).

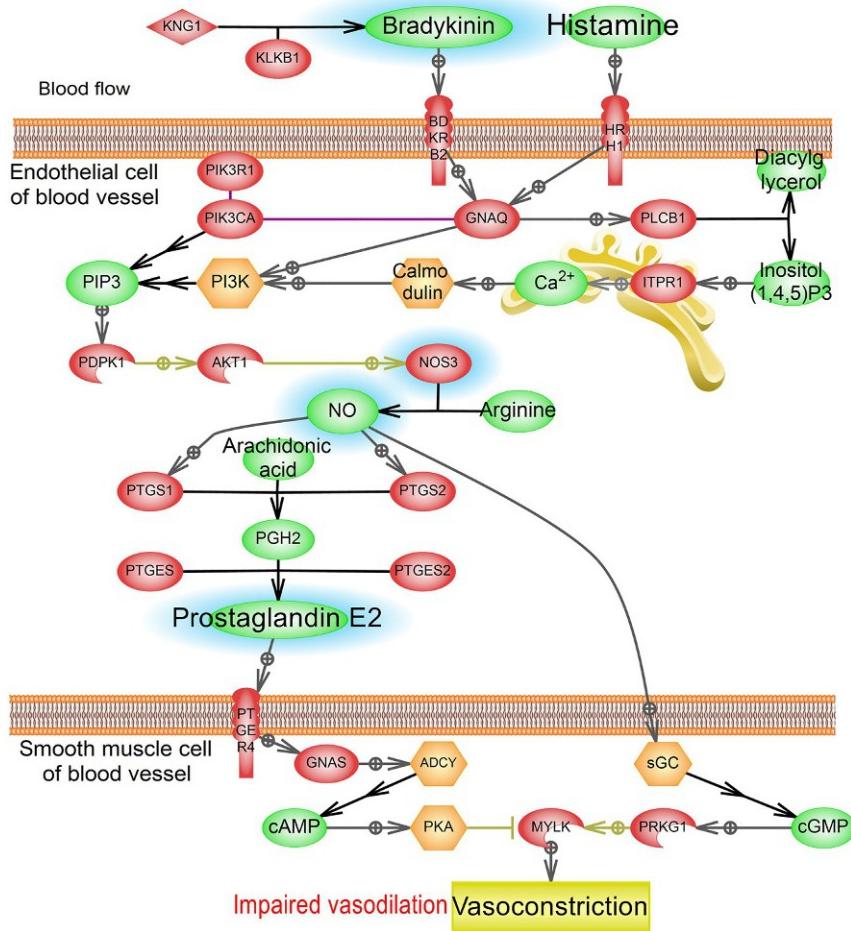


FIG. 7 Pathway 4: Endothelial cells repress vasoconstriction in hypertension.

## References

- Disease numbers # 145500 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code I10. Diseases of the circulatory system (I00-I99). (ICD-10, <https://icdlist.com>). ICD-11: disease code BA00/BA00.Z.
- Baudrand, R., Vaidya, A., 2018. The low-renin hypertension phenotype: genetics and the role of the mineralocorticoid receptor. *Int. J. Mol. Sci.* 19. <https://doi.org/10.3390/ijms19020546>.
- Botzer, A., Grossman, E., Moult, J., Unger, R., 2018. A system view and analysis of essential hypertension. *J. Hypertens.* <https://doi.org/10.1097/HJH.0000000000001680>.
- Burrello, J., Monticone, S., Buffolo, F., Tetti, M., Veglio, F., Williams, T.A., Mulatero, P., 2017. Is there a role for genomics in the management of hypertension? *Int. J. Mol. Sci.* 18, <https://doi.org/10.3390/ijms18061131>.
- Currie, G., Delles, C., 2016. Use of biomarkers in the evaluation and treatment of hypertensive patients. *Curr. Hypertens. Rep.* 18, 54. <https://doi.org/10.1007/s11906-016-0661-6>.
- Dbouk, H.A., Huang, C.-L., Cobb, M.H., 2016. Hypertension: the missing WNKs. *Am. J. Physiol. Renal Physiol.* 311, F16–F27. <https://doi.org/10.1152/ajprenal.00358.2015>.
- Delles, C., McBride, M.W., Graham, D., Padmanabhan, S., Dominiczak, A.F., 2010. Genetics of hypertension: from experimental animals to humans. *Biochim. Biophys. Acta* 1802, 1299–1308. <https://doi.org/10.1016/j.bbadi.2009.12.006>.
- Ehret, G.B., Caulfield, M.J., 2013. Genes for blood pressure: an opportunity to understand hypertension. *Eur. Heart J.* 34, 951–961. <https://doi.org/10.1093/eurheartj/ehs455>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Hamm, L.L., Hering-Smith, K.S., 2010. Pivotal role of the kidney in hypertension. *Am J Med Sci* 340, 30–32. <https://doi.org/10.1097/MAJ.0b013e3181e590f0>.
- Hattangady, N.G., Olala, L.O., Bollag, W.B., Rainey, W.E., 2012. Acute and chronic regulation of aldosterone production. *Mol. Cell. Endocrinol.* 350, 151–162. <https://doi.org/10.1016/j.mce.2011.07.034>.
- Kohan, D.E., Rossi, N.F., Inscho, E.W., Pollock, D.M., 2011. Regulation of blood pressure and salt homeostasis by endothelin. *Physiol. Rev.* 91, 1–77. <https://doi.org/10.1152/physrev.00060.2009>.
- Konukoglu, D., Uzun, H., 2017. Endothelial dysfunction and hypertension. *Adv. Exp. Med. Biol.* 956, 511–540. [https://doi.org/10.1007/5584\\_2016\\_90](https://doi.org/10.1007/5584_2016_90).
- Loirand, G., Pacaud, P., 2014. Involvement of Rho GTPases and their regulators in the pathogenesis of hypertension. *Small GTPases* 5, 1–10. <https://doi.org/10.4161/sgt.28846>.
- Murthy, M., Kurz, T., O'Shaughnessy, K.M., 2017. WNK signalling pathways in blood pressure regulation. *Cell. Mol. Life Sci.* 74, 1261–1280. <https://doi.org/10.1007/s00018-016-2402-z>.
- Rush, J.W.E., Aultman, C.D., 2008. Vascular biology of angiotensin and the impact of physical activity. *Appl. Physiol. Nutr. Metab. Appl. Nutr. Metab.* 33, 162–172. <https://doi.org/10.1139/H07-147>.
- Shah, B.H., Baukal, A.J., Chen, H.-D., Shah, A.B., Catt, K.J., 2006. Mechanisms of endothelin-1-induced MAP kinase activation in adrenal glomerulosa cells. *J. Steroid Biochem. Mol. Biol.* 102, 79–88. <https://doi.org/10.1016/j.jsbmb.2006.09.026>.
- Te Riet, L., van Esch, J.H.M., Roks, A.J.M., van den Meiracker, A.H., Danser, A.H.J., 2015. Hypertension: renin-angiotensin-aldosterone system alterations. *Circ. Res.* 116, 960–975. <https://doi.org/10.1161/CIRCRESAHA.116.303587>.
- Versari, D., Daghini, E., Virdis, A., Ghiaodoni, L., Taddei, S., 2009. Endothelium-dependent contractions and endothelial dysfunction in human hypertension. *Br. J. Pharmacol.* 157, 527–536. <https://doi.org/10.1111/j.1476-5381.2009.00240.x>.
- Yogi, A., Callera, G.E., Antunes, T.T., Tostes, R.C., Touyz, R.M., 2011. Transient receptor potential melastatin 7 (TRPM7) cation channels, magnesium and the vascular system in hypertension. *Circ. J. Off. J. Jpn. Circ. Soc.* 75, 237–245.

## CHAPTER

## 8.3

## Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a progressive disorder characterized by abnormally high blood pressure (hypertension) in the pulmonary artery, the blood vessel that carries blood from the heart to the lungs. PAH is a subtype of a broader condition known as pulmonary hypertension (PH) (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Several genetic abnormalities have been associated with heritable PAH, many of which are mutations in the genes that code for members of the TGFB family of receptors (BMPR2, ACVRL1, ENG). (*Ferri and Ferri, 2018*).

Pulmonary arterial hypertension occurs when most of the very small arteries in the lungs narrow in diameter leading to increased resistance to blood flow through the lungs. To overcome the increased resistance, blood pressure in the pulmonary artery and in the right ventricle of the heart rises, and PAH progresses. Ultimately the resulting increased blood pressure can damage the right ventricle of the heart.

PAH is subclassified as either familial (FPAH), idiopathic (IPAH), or acquired (APA). There are several known risk factors for PAH including connective tissue disorders, portal hypertension and liver cirrhosis, appetite-suppressant drugs (fenfluramine), hemoglobinopathies, infections, and genetics (*Ferri and Ferri, 2018*).

Various infections, including schistosomiasis and human immunodeficiency virus (HIV) as well as sickle cell disease, are the most common causes of general PAH worldwide, although pulmonary venous hypertension resulting from left ventricular failure and PAH related to chronic obstructive pulmonary disease (COPD) are more common causes of PAH in developed nations.

Different molecular mechanisms increase vasoconstriction and weaken vasodilation in PAH. Dysfunction of endothelial cells shifts the balance to vasoconstriction and is present in all types of PAH:

**Pathway 1.** *Endothelial cells stimulate vasoconstriction and thrombosis in pulmonary hypertension (Fig. 8).*

Signaling molecules synthesized in response to hypertension increase contractile activity of smooth muscle cells. High levels of smooth muscle cell contraction lead to artery lumen narrowing and a consequent increase in pulmonary vascular resistance.

**Pathway 2.** *The intense proliferation and contractile activity of smooth muscle cells in pulmonary hypertension (Fig. 9).*

Several forms of FPAH are described.

**Pathway 3.** *Increased proliferation of pulmonary artery smooth muscle cells (PASMCs) in familial forms of pulmonary hypertension (Fig. 10).*

## Key cellular contributors and processes

Endothelial cells

Cell

Endothelial cells (ECs) are single-layered cells that line the inside of blood and lymph vessels and mediate the selective movement of substances and cells between the bloodstream and surrounding cells.

Pulmonary artery smooth muscle cells

Cell

Pulmonary artery smooth muscle cells (PASMCs) are nonstriated muscular cells of the pulmonary artery wall localized in arterial media layer (middle coat of the artery).

Vasoconstriction

Process

Vasoconstriction is the narrowing of blood vessels caused by the contraction of smooth muscles in their wall. Vasoconstriction decreases the blood flow through the vessels and increases the blood pressure.

Vasodilation

Process

Vasodilation is the widening of blood vessels caused by the relaxation of smooth muscle cells in their walls. Vasodilation increases the blood flow through the vessels and decreases the blood pressure.

## Pathway 1

### **Endothelial cells stimulate vasoconstriction and thrombosis in pulmonary hypertension ([Fig. 8](#))**

#### **Incoming signals**

The endothelium plays a significant role in the maintenance of vascular tone via the production and release of vasodilators (e.g., nitric oxide (NO) and prostacyclin) and vasoconstrictors (e.g., endothelin (EDN1) and serotonin). The dysfunction of endothelial cells in PAH is characterized by decreased production of vasodilators and increased production of vasoconstrictors.

The level of angiopoietin 1 (ANGPT1) secreted by vascular smooth muscle cells (SMCs) is higher in patients with some forms of PAH. There is also evidence indicating that the levels of the vasoconstrictor thromboxane A<sub>2</sub>, synthesized by platelets or other cells, are elevated in patients with PAH.

#### **Outcome effects**

The decreased production of NO and prostacyclin by endothelial cells provokes vasoconstriction. Increased secretion of the EDN1, vascular endothelial growth factor A (VEGFA), and serotonin causes endothelial cell proliferation, which in turn leads to artery lumen narrowing and a predisposition to thrombosis.

#### **Signaling**

Under healthy conditions, nitric oxide synthase 3 (NOS3) produces NO from arginine and oxygen. Upon entering the SMCs, NO activates soluble guanylate cyclase (sGC), which in turn produces cyclic guanosine monophosphate (cGMP), an inhibitor of vasoconstriction (see [Pathway 2](#)). Patients with PAH have reduced NO bioavailability, which may be caused by decreased expression levels of NOS3 (not shown), inhibition of NOS3 enzymatic activity by asymmetric dimethylarginine and caveolin 1 (CAV1), or by the inactivation of NO by superoxide. Increased activity of arginase 2 (ARG2) may reduce NO synthesis by competing with NOS3 for available arginine. Local hyperproduction of reactive oxygen species (ROS) in endothelial cells by the NADPH oxidases, such as cytochrome *b*-245 beta chain (CYBB) and NADPH oxidase 4 (NOX4), and by xanthine dehydrogenase (XDH) also plays an important role in regulating NO levels.

The decreased production of another vasodilator (prostacyclin) normally secreted by endothelial cells (ECs) has been observed in PAH. There is a hypothesis that an imbalance between a high level of thromboxane A2 and a low level of prostacyclin follows shear stress and hypoxia and promotes increased secretion of vasoconstrictors (Christman et al., 1992). Prostaglandin I2 synthase (PGIS) may transform prostaglandin H2 to thromboxane A2 and prostacyclin. Prostacyclin functions in an opposite manner to thromboxane 2; it has a relaxing effect on the vessels and acts as an anticoagulant.

ANGPT1 is secreted by vascular SMCs and pericytes at high levels in most forms of nonfamilial PAH. ANGPT1 acts through the endothelial-specific tyrosine kinase receptor (TEK) to stimulate ECs to produce and secrete both serotonin and EDN1. ANGPT1-dependent increase in levels of tryptophan hydroxylase 1 (TPH1) and endothelin-converting enzyme 1 (ECE1) was detected in PAH. TPH1 is a rate-limiting enzyme involved with serotonin synthesis. However, the exact mechanism of ANGPT1 action is not clear yet.

EDN1, thromboxane A2, and serotonin operate together with growth factors (such as VEGFA), WNT family members, and cytokines (not shown) to promote EC proliferation. The G protein-coupled receptor signaling pathways, including PI3K signaling (not shown) and SRC signaling, are the most critical ways of activating EC proliferation. Higher levels of Ras homolog gene family, member A (RHOA) expression in PAH may contribute to NFkB transcription complex activation. RHOA and NFkB together induce cyclin D1 (CCND1)-dependent proliferation of ECs, and they regulate the expression of other proteins including serpin family E member 1 (SERPINE1), thromboplastin (F3), selectin P (SELP), and thrombomodulin (THBD) that may induce blood clotting to facilitate thrombosis.

The activated RHOA/ROCK1 pathway promotes cellular actin filament assembly, EC contraction, and increased blood vessel permeability leading to disease progression (Connolly and Aaronson, 2011; de Jesus Perez et al., 2009; Dewachter et al., 2006; Lourenço et al., 2012; MacLean and Dempsie, 2009; Morrell et al., 2009; Rabinovitch, 2008; Seeger and Pullamsetti, 2013; Tabima et al., 2012).

## II. Human disease pathways

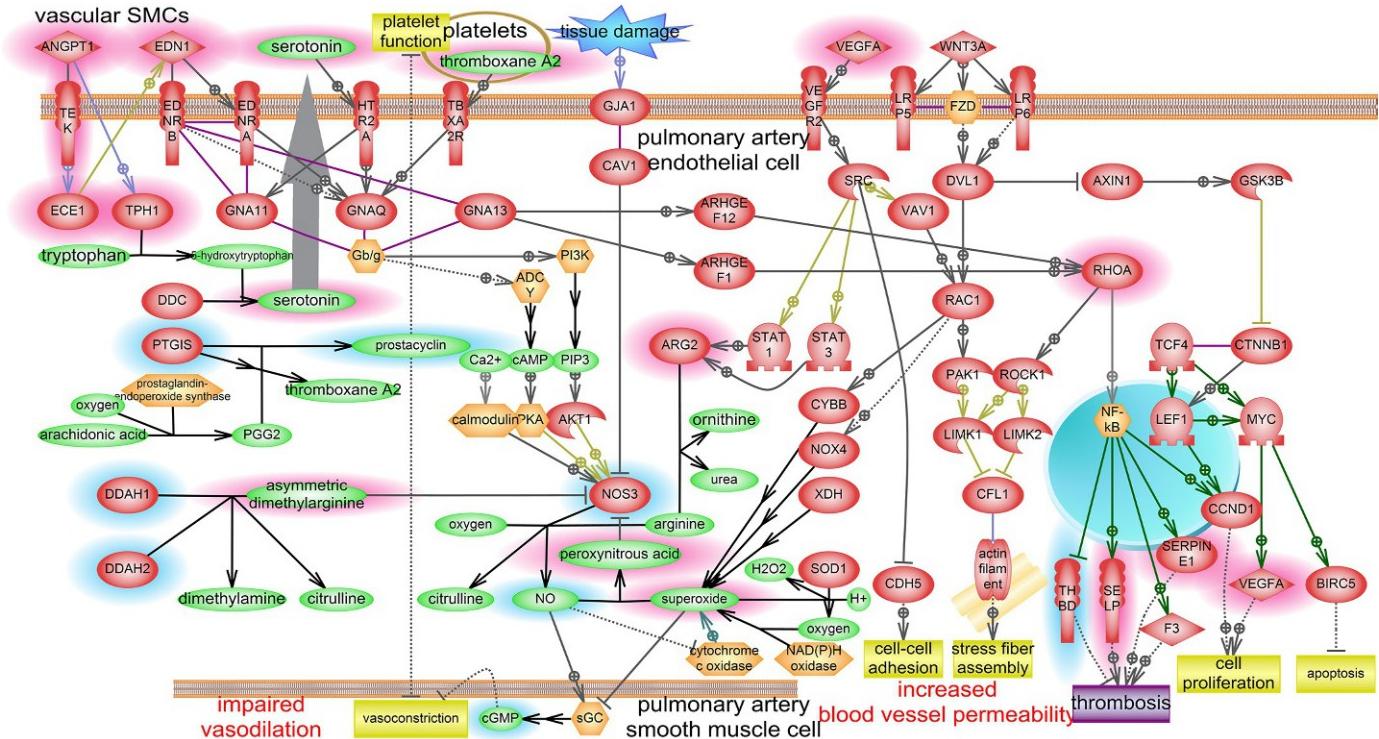


FIG. 8 Pathway 1: Endothelial cells stimulate vasoconstriction and thrombosis in pulmonary hypertension.

## Pathway 2

### The intense proliferation and contractile activity of smooth muscle cells in pulmonary hypertension ([Fig. 9](#))

#### Incoming signals

Vasoconstrictors and other signals from endothelial cells induce contraction and the proliferation of pulmonary artery smooth muscle cells (PASMCs).

Growth factors including epidermal growth factor (EGF), platelet-derived growth factor subunits A and B (PDGFA and PDGFB), and insulin-like growth factor 1 (IGF1) are all overexpressed in PAH, and they play a role in the stimulation of PASMCs in PAH.

Levels of the hormone angiotensin II and the extracellular protein tenascin-C (TNC), both associated with vascular remodeling, are elevated in patients with PAH.

#### Outcome effects

Enhanced PASMC proliferation and contractility result in artery wall thickening and lumen narrowing, which are the primary factors leading to increased pulmonary vascular resistance and the development of pulmonary hypertension.

#### Signaling

Broad numbers of vasoconstrictors, including EDN1, thromboxane A2, and serotonin, produced by endothelial cells may stimulate G protein-coupled receptor signaling that leads to RHOA activation. This in turn activates ROCK1. ROCK1 inhibits myosin light chain phosphatase (MLCP) leading to the phosphorylation of myosin light chain 12B (MYL12B) resulting in vascular smooth muscle cell contraction. ROCK1 is also involved in actin cytoskeleton dynamics through the phosphorylation of LIMK1/2, forming F-actin stress fibers.

On the other hand, activation of the RHOA/ROCK1 pathway increases PASMC proliferation through MAPK1/MAPK3 signaling. Phosphorylated MAPK1/MAPK3 activates proliferative transcription factors such as GATA binding protein 4 (GATA4), early growth response protein 1 (EGR1), and ETS transcription factor (ELK1). Growth factors stimulate proliferation and assembly of the actin cytoskeleton via calcium mobilization, RHOA and MAPK1/MAPK3 activation, and other cellular responses (only SHC1-dependent signaling is shown for simplicity).

High levels of serotonin have both contractile and proliferative effects on PASMCs. The contractile action of serotonin is mediated via the activation of serotonin receptors and G protein-mediated signaling. The proliferative effect of serotonin requires the solute carrier family 6 member 4 (SLC6A4) transporter and monoamine oxidase A (MAOA). The breakdown of serotonin by MAOA results in the generation of ROS that are responsible for the translocation of phosphorylated MAPK1/MAPK3 into the nucleus.

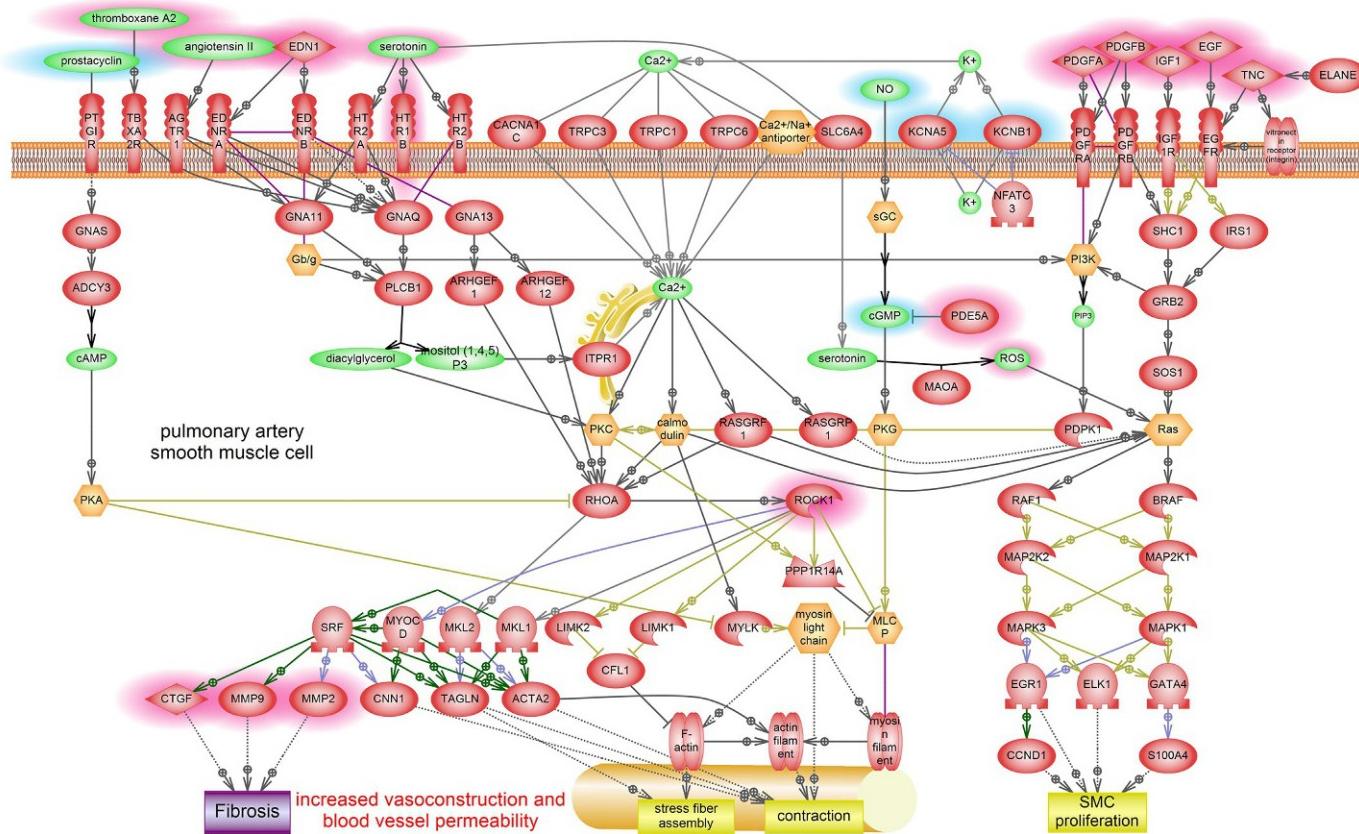
Cytosolic  $\text{Ca}^{2+}$  concentration is the essential determinant of contraction and proliferation of PASMCs. The cytosolic  $\text{Ca}^{2+}$  concentration can be increased by  $\text{Ca}^{2+}$  influx through the voltage-dependent  $\text{Ca}^{2+}$  channels calcium voltage-gated channel subunit alpha-1C (CACNA1C), and transient receptor potential channels 1, 3, and 6 (TRPC1/TRPC3/TRPC6) or  $\text{Ca}^{2+}$  may be released from stores within the sarcoplasmic reticulum. In PAH, TRPC3/TRPC6 channels were expressed at high levels, while the potassium ( $\text{K}^+$ ) channels potassium voltage-gated channels subfamily A member 5 and subfamily B member 1 (KCNA5 and KCNB1) were expressed at low levels. Decreased activity of  $\text{K}^+$  channels leads to membrane depolarization and contributes to a rise in the cytosolic  $\text{Ca}^{2+}$  concentration. Calcium stimulates PASMC contraction and proliferation by activating myosin light chain kinase (MYLK) and MAPK1/MAPK3 signaling.

A decrease in the levels of NO and prostacyclin secreted from epithelium inhibits myosin light chain and PASMC contraction.

An increase in the levels of TNC, normally expressed in the embryonic program, may signify the initiation of the vascular remodeling process.

Specific nuclear transcription factors such as serum response factor (SRF), myocardin (MYOCD), and myocardin-like proteins 1 and 2 (MKL1/2) induce expression of the metalloproteinases 2 and 9 (MMP2/MMP9), transgelin (TAGLN), and calponin-1 (CNN1) genes, which together influence the contractile phenotype in PASMCs and lead to fibrosis development (Connolly and Aaronson, 2011; Li et al., 2009; Lourenço et al., 2012; MacLean and Dempsey, 2009; Morrell et al., 2009; Rabinovitch, 2008; Seeger and Pullamsetti, 2013; Tabima et al., 2012).

## II. Human disease pathways



**FIG. 9** Pathway 2: The intense proliferation and contractile activity of smooth muscle cells in pulmonary hypertension.

## Pathway 3

### Increased proliferation of PASMCs in familial forms of pulmonary hypertension ([Fig. 10](#))

#### Incoming signals

Familial pulmonary arterial hypertension (FPAH) is associated with mutations in the genes encoding bone morphogenetic protein receptor type 2 (*BMPR2*), activin A receptor-like type 1 (*ACVRL1*), endoglin (*ENG*), and some other genes. Mutations in the *BMPR2* gene have been identified in over 75% of patients with FPAH and in 10%–40% of patients with idiopathic PAH. Less common mutations in the *ACVRL1* and *ENG* genes are associated with both FPAH and hereditary hemorrhagic telangiectasia. There may also be mutations in the *SMAD9* and bone morphogenetic protein receptor type 1B (*BMPR1B*) genes, although they are rare.

#### Outcome effects

BMP and transforming growth factor beta 1 (TGFB1) signalings maintain cellular specialization and slow down cell proliferation. In FPAH and IPAH the BMP and TGFB1 signaling pathways are not fully activated, and the balance between PASMC proliferation and differentiation shifts toward proliferation. Excessive PASMC proliferation leads to pulmonary artery wall thickening, lumen narrowing, elevation of pulmonary vascular resistance, and the development of pulmonary hypertension.

#### Signaling

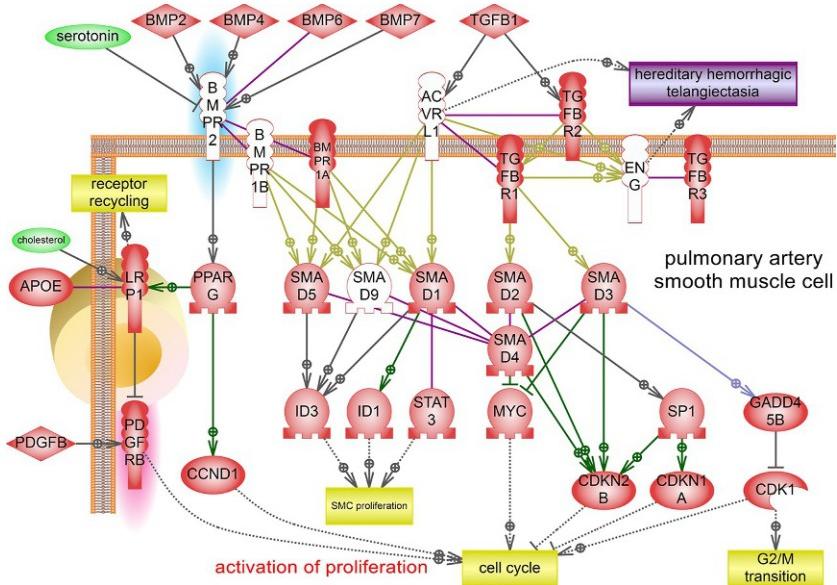
The bone morphogenetic protein receptor type 1A, 1B, and 2 (*BMPR1A*, *BMPR1B*, and *BMPR2*) proteins form a complex that phosphorylates cytosolic SMAD1/SMAD5/SMAD9. When activated the SMAD proteins interact with a nuclear chaperone, SMAD4, and are translocated to the nucleus to initiate transcription of their target genes. TGFB1 signaling acts through SMAD 2 and SMAD3 and also enrolls SMAD4 as its coactivator. Normally, BMP and TGFB1 signaling leads to activation of the cell cycle regulators cyclin-dependent kinase inhibitors 1A and 2B (*CDKN1A* and *CDKN2B*) and cyclin-dependent kinase 1 (*CDK1*) as well as other nuclear transcription factors.

Receptor loss-of-function mutations in PAH impair the expression of proteins dependent on the SMADs and shifts the balance toward PASMC proliferation (see [Pathway 2](#)).

A mutant *BMPR2* gene product also can stimulate PASMC proliferation via the SMADs by an independent mechanism. Apolipoprotein E (APOE)

in healthy cells binds to low-density lipoprotein receptor-related protein 1 (LRP1), thereby initiating retrograde endocytosis to internalize and inactivate platelet-derived growth factor receptor B (PDGFRB). A mutant *BMPR2* gene product with decreased activity may lead to PDGFRB over-representation on cell membranes, which in turn leads to the activation of PDGFRB signaling (not shown) and cell proliferation.

Serotonin-mediated signaling is associated with the development of PAH independently of mutations in the *BMPR2* gene. Serotonin may antagonize the *BMPR2* pathway, and it facilitates proliferation ([Eickelberg and Morty, 2007](#); [Lourenço et al., 2012](#); [Machado et al., 2009](#); [MacLean and Dempsie, 2009](#)).



**FIG. 10** Pathway 3: Increased proliferation of pulmonary artery smooth muscle cells (PASMCs) in familial forms of pulmonary hypertension.

## References

- Disease numbers # 178600, # 600376, # 615343, # 615344, # 615342 (and many others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code I27.0/I27.2. Diseases of the circulatory system (I00-I99). (ICD-10, <https://icdlist.com>). ICD-11: disease code BB01.
- Christman, B.W., McPherson, C.D., Newman, J.H., King, G.A., Bernard, G.R., Groves, B.M., Loyd, J.E., 1992. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N. Engl. J. Med.* 327, 70–75. <https://doi.org/10.1056/NEJM199207093270202>.
- Connolly, M.J., Aaronson, P.I., 2011. Key role of the RhoA/Rho kinase system in pulmonary hypertension. *Pulm. Pharmacol. Ther.* 24, 1–14. <https://doi.org/10.1016/j.pupt.2010.09.001>.
- de Jesus Perez, V.A., Alastalo, T.-P., Wu, J.C., Axelrod, J.D., Cooke, J.P., Amieva, M., Rabinovitch, M., 2009. Bone morphogenetic protein 2 induces pulmonary angiogenesis via Wnt-beta-catenin and Wnt-RhoA-Rac1 pathways. *J. Cell Biol.* 184, 83–99. <https://doi.org/10.1083/jcb.200806049>.
- Dewachter, L., Adnot, S., Fadel, E., Humbert, M., Maitre, B., Barlier-Mur, A.-M., Simonneau, G., Hamon, M., Naeije, R., Eddahibi, S., 2006. Angiopoietin/Tie2 pathway influences smooth muscle hyperplasia in idiopathic pulmonary hypertension. *Am. J. Respir. Crit. Care Med.* 174, 1025–1033. <https://doi.org/10.1164/rccm.200602-304OC>.
- Eickelberg, O., Morty, R.E., 2007. Transforming growth factor beta/bone morphogenic protein signaling in pulmonary arterial hypertension: remodeling revisited. *Trends Cardiovasc. Med.* 17, 263–269. <https://doi.org/10.1016/j.tcm.2007.09.003>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Li, X., Zhang, X., Leathers, R., Makino, A., Huang, C., Parsa, P., Macias, J., Yuan, J.X.-J., Jamieson, S.W., Thistlethwaite, P.A., 2009. Notch3 signaling promotes the development of pulmonary arterial hypertension. *Nat. Med.* 15, 1289–1297. <https://doi.org/10.1038/nm.2021>.
- Lourenço, A.P., Fontoura, D., Henriques-Coelho, T., Leite-Moreira, A.F., 2012. Current pathophysiological concepts and management of pulmonary hypertension. *Int. J. Cardiol.* 155, 350–361. <https://doi.org/10.1016/j.ijcard.2011.05.066>.
- Machado, R.D., Eickelberg, O., Elliott, C.G., Geraci, M.W., Hanaoka, M., Loyd, J.E., Newman, J.H., Phillips, J.A., Soubrier, F., Trembath, R.C., Chung, W.K., 2009. Genetics and genomics of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 54, S32–S42. <https://doi.org/10.1016/j.jacc.2009.04.015>.
- MacLean, M.R., Dempsie, Y., 2009. Serotonin and pulmonary hypertension—from bench to bedside? *Curr. Opin. Pharmacol.* 9, 281–286. <https://doi.org/10.1016/j.coph.2009.02.005>.
- Morrell, N.W., Adnot, S., Archer, S.L., Dupuis, J., Jones, P.L., MacLean, M.R., McMurtry, I.F., Stenmark, K.R., Thistlethwaite, P.A., Weissmann, N., Yuan, J.X.-J., Weir, E.K., 2009. Cellular and molecular basis of pulmonary arterial hypertension. *J. Am. Coll. Cardiol.* 54, S20–S31. <https://doi.org/10.1016/j.jacc.2009.04.018>.
- Rabinovitch, M., 2008. Molecular pathogenesis of pulmonary arterial hypertension. *J. Clin. Invest.* 118, 2372–2379. <https://doi.org/10.1172/JCI33452>.
- Seeger, W., Pullamsetti, S.S., 2013. Mechanics and mechanisms of pulmonary hypertension—conference summary and translational perspectives. *Pulm. Circ.* 3, 128–136.
- Tabima, D.M., Frizzell, S., Gladwin, M.T., 2012. Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Radic. Biol. Med.* 52, 1970–1986. <https://doi.org/10.1016/j.freeradbiomed.2012.02.041>.

## CHAPTER

## 8.4

## Hypertrophic cardiomyopathy

Mutations in one of several genes that regulate myocyte architecture can cause familial hypertrophic cardiomyopathy (HCM).

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant myocardial disorder characterized by disorganized myocyte architecture and marked thickening (hypertrophy) of the left ventricular wall ( $>15$  mm), without dilation, not explained by another cardiac or systemic disorder. The interventricular septum is the most common site of enlargement, though hypertrophy may involve other focal regions or may be concentric. (*Ferri and Ferri, 2018*).

Myosin heavy chain 7 (*MYH7*), cardiac myosin-binding protein C (*MYBPC3*), and cardiac troponin T (*TNNT2*) are the three most common causal genes for HCM and collectively account for approximately 75% of all HCM cases.

Other genes, including some that have not been identified, may also be involved in this condition.

The proteins produced from the genes associated with familial hypertrophic cardiomyopathy play important roles in heart muscle contraction and forming the sarcomeres, which are the basic units of muscle contraction (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Mutations in sarcomeric proteins cause disorganization of myocyte architecture and impaired sarcomere assembly:

**Pathway 1.** Sarcomere disorganization and intracellular calcium overload (Fig. 11).

Compensatory cardiomyocyte hypertrophy and fibrosis develop in response to reduced cardiomyocyte contractility.

**Pathway 2.** Cardiomyocyte hypertrophy (Fig. 12).

## Key cellular contributors and processes

### Cardiomyocyte hypertrophy

#### Process

Cardiac hypertrophy is the adaptive enlargement of cardiomyocytes in response to pressure or volume stress, which leads to the thickening of the heart muscle.

### Sarcomere

#### Anatomic structure

Sarcomere is the contractile unit of striated muscle myofibrils that consists of a large number of parallel actin (thin) and myosin (thick) protein filaments. In the sarcomere, actin filaments at their plus ends are tethered to structures located at the lateral ends of each sarcomere called Z discs, and myosin is bound to the M line in the middle of the sarcomere. Additional proteins, such as nebulin and titin, are involved in maintaining sarcomere structure and stability.

## Pathway 1

### Sarcomere disorganization and intracellular calcium overload [\(Fig. 11\)](#)

#### Incoming signals

Mutations in more than 20 genes may induce HCM. Most of these genes encode sarcomeric proteins; however, genes encoding nonsarcomeric proteins may also be involved.

Sarcomeres are the basic units of muscle contraction made up of thick and thin protein filaments. The overlapping thick and thin filaments attach to each other temporarily and release, allowing the filaments to move relative to one another so that muscles can contract.

#### Outcome effects

Mutations in the genes encoding sarcomeric and nonsarcomeric proteins and intracellular  $\text{Ca}^{2+}$  overload lead to impaired sarcomere assembly and impaired myofibrillar contraction. This leads to compensatory cardiomyocyte hypertrophy or enlargement of cardiomyocytes and a thickening of the heart muscle.

#### Signaling

The sarcomeric protein encoding genes cardiac troponin I3 (*TNNI3*), cardiac troponin C1 (*TNNC1*), cardiac alpha-actin 1 (*ACTC1*), tropomysin 1 (*TPM1*), myosin light chain 3 (*MYL3*), myosin light chain 2 (*MYL2*), and myosin heavy chain 6 (*MYH6*) are necessary for the proper function of sarcomere filaments. All these genes may be mutated in HCM.

In addition, HCM-causing mutations have been identified in several genes encoding Z disc proteins including titin-cap (*TCAP*), myozenin 2 (*MYOZ2*), cysteine and glycine-rich protein 3 (*CSRP3*), and ankyrin repeat domain 1 (*ANKRD1*). Additional mutations have been observed in genes that encode proteins connecting the plasma membrane with sarcomeres such as vinculin (*VCL*), junctophilin 2 (*JPH2*), and caveolin 3 (*CAV3*). Titin (*TTN*), a protein spanning the distance from the Z- to M-discs, may also be mutated.

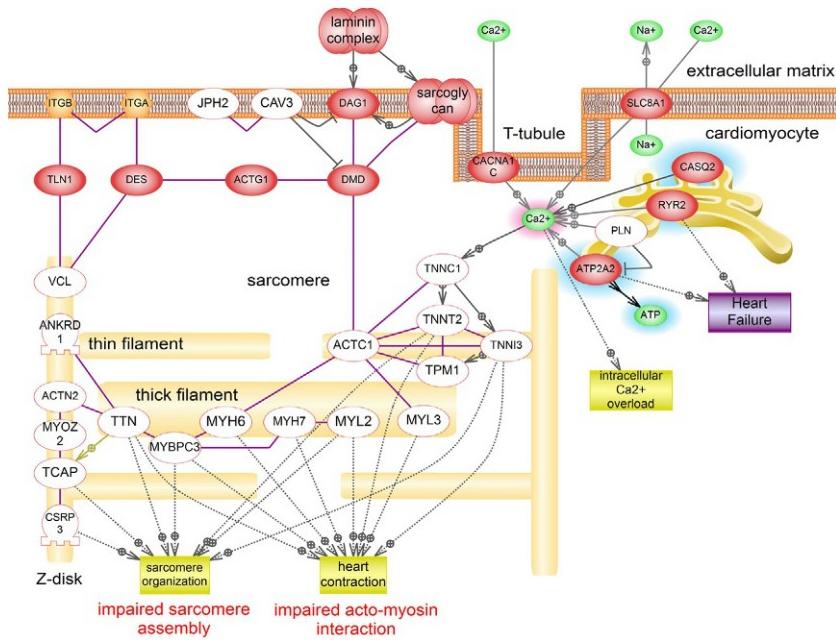
Mutations in sarcomeric and nonsarcomeric proteins lead to impaired sarcomere assembly and impaired myofibrillar contraction.

$\text{Ca}^{2+}$  also plays an important role in the pathogenesis of HCM.

Normally during the propagation of an action potential,  $\text{Ca}^{2+}$  enters the cardiomyocyte through the calcium voltage-gated channel subunit alpha-1C (*CACNA1C*) channel that is responsible for  $\text{Ca}^{2+}$  influx. To

a lesser extent, it also enters via SLC8A1 ( $\text{Na}^+/\text{Ca}^{2+}$  exchanger solute carrier family 8 member A1), thereby triggering  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR) through ryanodine receptor 2 (RYR2). This  $\text{Ca}^{2+}$  binds TNNTC1 and activates the myofilaments during systole leading to myocardial contraction. During diastole,  $\text{Ca}^{2+}$  is taken back up into the sarcoplasmic reticulum by ATP2A2 ( $\text{Ca}^{2+}$  transporting sarcoplasmic/endoplasmic reticulum ATPase 2) and is pumped out of the cells by the solute carrier family 8 member 1 (SLC8A1) protein that results in muscular relaxation.

Mutated sarcomeric proteins use more adenosine triphosphate (ATP) to produce the same force of contraction. Therefore those mutations compromise  $\text{Ca}^{2+}$  reuptake by the ATP2A2 transporter into the SR, so more  $\text{Ca}^{2+}$  becomes available inside the cells. Finally, intracellular  $\text{Ca}^{2+}$  overload also leads to compensatory cardiomyocyte hypertrophy (Ashrafi et al., 2011; Frey et al., 2011; Harvey and Leinwand, 2011; Maron et al., 2012; Maron and Maron, 2013; Seidman and Seidman, 2011).



**FIG. 11** Pathway 1: Sarcomere disorganization and intracellular calcium overload.

## Pathway 2

### Cardiomyocyte hypertrophy (Fig. 12)

#### Incoming signals

Mutations in genes that encode sarcomeric and nonsarcomeric proteins lead to impaired sarcomere assembly and impaired cardiomyocyte contractility. Angiotensin II (AGT), endothelin-1 (EDN1), and IL-6 are released in response to reduced contractility and mediate contractile adaptation through the release of increased levels of  $\text{Ca}^{2+}$  from the SR.

#### Outcome effects

The final morphological events resulting from these molecular mechanisms are prominent myocardial hypertrophy, myocardial disarray, and increased interstitial fibrosis, which together lead to diastolic dysfunction and heart failure.

#### Signaling

An increase in intracellular  $\text{Ca}^{2+}$  activates the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase (CAMK) pathways to trigger the activation of the nuclear transcription factors myocyte enhancer factor 2A (MEF2A), GATA binding protein 4 (GATA4), and nuclear factor of activated T cells, cytoplasmic 1 (NFATC1), which induce hypertrophy through the expression of contractile proteins. They also induce the expression of fetal isoforms of several proteins including natriuretic peptides A and B (NPPA/NPPB) and skeletal muscle alpha-actin 1 (ACTA1). AGT, EDN1, TNF, and IL-6 activate other nuclear transcription factors including NFkB, STAT, JUN/FOS, and SMAD and thereby drive increased levels of tissue inhibitors of metalloproteinase 1 and 2 (TIMP1/TIMP2) and matrix metalloproteinases 2 and 9 (MMP2/MMP9) transcription, which in turn induces remodeling of the extracellular matrix. In addition, increased myocardial fibrosis occurs due to the increased production of transforming growth factor beta 1 (TGFB1) by cardiomyocytes and increased fibroblast proliferation and enhanced fibroblast function (Akazawa and Komuro, 2003; Bernardo et al., 2010; Cambronero et al., 2009; Frey et al., 2011; Harvey and Leinwand, 2011).

## II. Human disease pathways

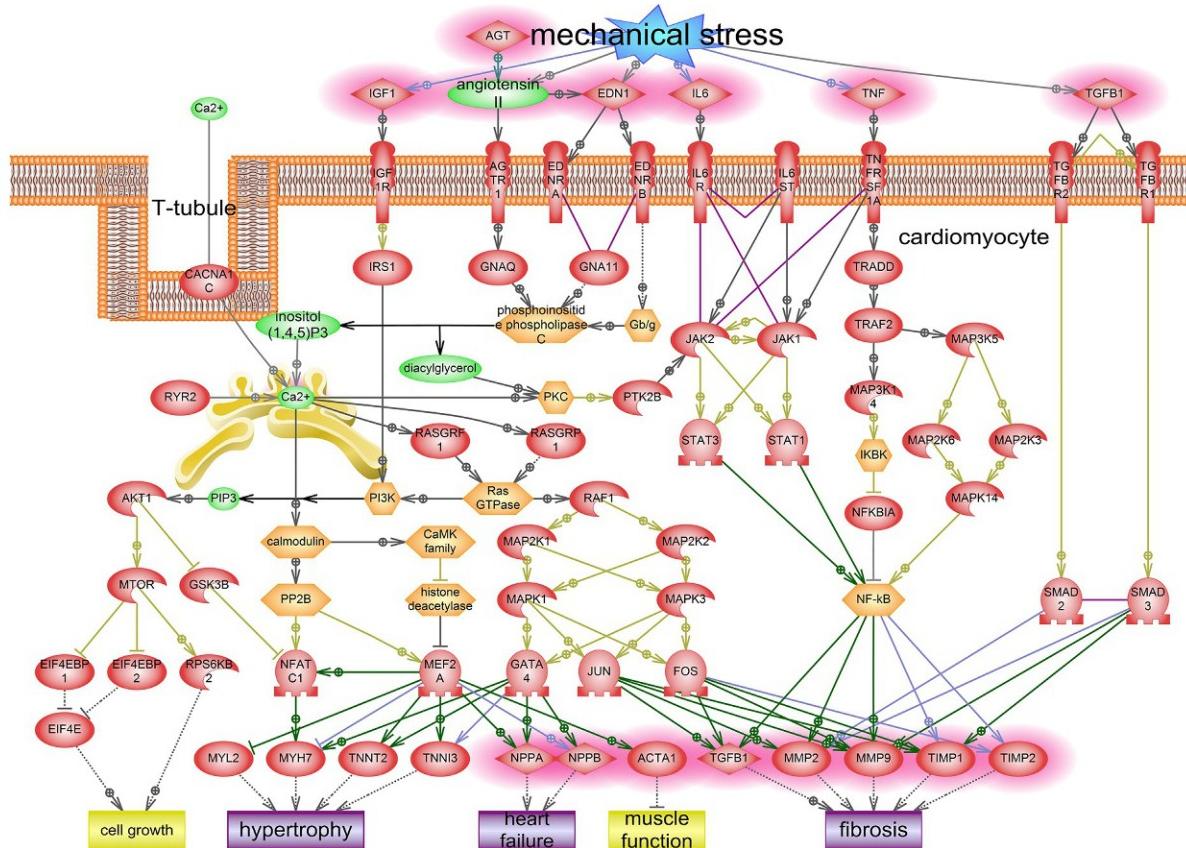


FIG. 12 Pathway 2: Cardiomyocyte hypertrophy.

## References

- Disease numbers # 192600, # 115195, # 600858 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code I42.2. Diseases of the circulatory system (I00-I99). (ICD-10, <https://icdlist.com>). ICD-11: disease code BC43.1.
- Akazawa, H., Komuro, I., 2003. Roles of cardiac transcription factors in cardiac hypertrophy. *Circ. Res.* 92, 1079–1088. <https://doi.org/10.1161/01.RES.0000072977.86706.23>.
- Ashrafian, H., McKenna, W.J., Watkins, H., 2011. Disease pathways and novel therapeutic targets in hypertrophic cardiomyopathy. *Circ. Res.* 109, 86–96. <https://doi.org/10.1161/CIRCRESAHA.111.242974>.
- Bernardo, B.C., Weeks, K.L., Pretorius, L., McMullen, J.R., 2010. Molecular distinction between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies. *Pharmacol. Ther.* 128, 191–227. <https://doi.org/10.1016/j.pharmthera.2010.04.005>.
- Cambrónero, F., Marín, F., Roldán, V., Hernández-Romero, D., Valdés, M., Lip, G.Y.H., 2009. Biomarkers of pathophysiology in hypertrophic cardiomyopathy: implications for clinical management and prognosis. *Eur. Heart J.* 30, 139–151. <https://doi.org/10.1093/eurheartj/ehn538>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Frey, N., Luedde, M., Katus, H.A., 2011. Mechanisms of disease: hypertrophic cardiomyopathy. *Nat. Rev. Cardiol.* 9, 91–100. <https://doi.org/10.1038/nrcardio.2011.159>.
- Harvey, P.A., Leinwand, L.A., 2011. The cell biology of disease: cellular mechanisms of cardiomyopathy. *J. Cell Biol.* 194, 355–365. <https://doi.org/10.1083/jcb.201101100>.
- Maron, B.J., Maron, M.S., 2013. Hypertrophic cardiomyopathy. *Lancet* 381, 242–255. [https://doi.org/10.1016/S0140-6736\(12\)60397-3](https://doi.org/10.1016/S0140-6736(12)60397-3).
- Maron, B.J., Maron, M.S., Semsarian, C., 2012. Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J. Am. Coll. Cardiol.* 60, 705–715. <https://doi.org/10.1016/j.jacc.2012.02.068>.
- Seidman, C.E., Seidman, J.G., 2011. Identifying sarcomere gene mutations in hypertrophic cardiomyopathy: a personal history. *Circ. Res.* 108, 743–750. <https://doi.org/10.1161/CIRCRESAHA.110.223834>.

## CHAPTER

## 8.5

## Arrhythmogenic right ventricular dysplasia (ARVD)

Arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVC/D, ARVD) can result from mutations in at least eight genes. Many of these genes are involved in the function of desmosomes, which are structures that attach heart muscle cells to one another.

Arrhythmogenic right ventricular dysplasia (ARVD) is a cardiomyopathy characterized by replacement of the normal myocardium with fibrofatty tissue, mainly of the right ventricle but also occasionally with involvement of the left ventricle. It is defined clinically by palpitations and syncope and potentially life-threatening ventricular arrhythmias. (*Ferri and Ferri, 2018*).

Up to half of all cases of ARVC/D appear to run in families. Most familial cases of the disease have an autosomal dominant pattern of inheritance, which means one copy of an altered gene in each cell is sufficient to cause the disorder (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Disruption of cell-to-cell junctions in cardiomyocytes due to mutations in several genes can be the cause of at least some cases of ARVD:

**Pathway 1.** *Adherens junction dysfunction in cardiomyocytes in ARVD (Fig. 13).*

**Pathway 2.** *Desmosome dysfunction in cardiomyocytes in ARVD (Fig. 14).* There are mutations in other genes not involved in adherens junctions or desmosomes that can cause ARVD.

**Pathway 3.** *Cardiomyocyte dysfunction unrelated to cell junctions in ARVD (Fig. 15).*

## Key cellular contributors and processes

Adherens junctions

Anatomic structure

Adherens junctions (AJs) are a type of intercellular junctions composed of the transmembrane protein E-cadherin and intracellular components, such as  $\beta$ - and  $\alpha$ -catenins, plakoglobin, and others. In AJs the cytoplasmic side of E-cadherin is connected to actin cytoskeleton through catenins. AJs provide maturation, stability, and plasticity of the cell-cell contact.

Cadherin

Protein or gene

Cadherins are a family of transmembrane calcium-dependent cell-cell adhesion molecules. Cadherins provide stability to the cell-cell contact and regulate its formation.

Cardiomyocyte

Cell

Cardiomyocytes are the principal muscular cells that make up the heart muscle and are responsible for generating the contractile force.

Cell-cell adhesion

Process

Cell-cell adhesion (intercellular adhesion) is a biological process by which cells form attachments to other cells via specialized cell adhesion molecules. The intercellular adhesion is a fundamental process underlying the formation of multicellular organisms. The major types of cell-cell adhesion include adherens junctions, tight junctions, and desmosomes.

Desmosome

Anatomic structure

Desmosomes are a type of intercellular junctions mediated by desmosomal cadherins bound on their intracellular side to intermediate filament (keratin) cytoskeleton through the cytoplasmic plaque proteins, plakoglobin and plakophilins, and other proteins. Desmosomes help withstand mechanical forces and participate in cell signaling.

## Pathway 1

### Adherens junction dysfunction in cardiomyocytes in ARVD [\(Fig. 13\)](#)

#### Incoming signals

Adherens junctions are cell-cell junctions that in cardiomyocytes are typically located more basally than tight junctions. Adherens junctions are composed of a complex of specialized proteins. Mutations in the genes encoding catenin alpha-3 (*CTNNA3*), junction plakoglobin (*JUP*) and plakophilin 2 (*PKP2*) cause instability of the adherens junctions between cardiomyocytes.

#### Outcome effects

Unstable contacts between cardiomyocytes due to mutations of the supportive proteins in adherens junctions provoke cardiomyocyte detachment, impair mechanical junctions and intercellular electrical conductivity, and probably can lead to heart arrhythmias.

#### Signaling

Adherens junctions contain cadherins, a family of transmembrane proteins that form homodimers in a calcium-dependent manner with other cadherin molecules on adjacent cells. The cytoplasmic face of the cadherins is linked to the actin cytoskeleton within the cardiomyocyte. In the adherens junction complex, the *JUP* protein binds to the catenin-binding region of cadherins. Catenins alpha-1 and alpha-2 (*CTNNA1/CTNNA2*) bind to the cadherins indirectly via catenin beta-1 (*CTNNB1*) or *JUP* and thereby link the actin cytoskeleton with cadherins. *CTNNA3* is encoded by a recently discovered gene that is associated with ARVD and probably can contribute to the pathogenesis of the disease. This gene encodes catenin alpha-3 that binds to plakophilin 2 (*PKP2*) and *JUP* and thereby contributes to the formation of the adherens junctions ([Campuzano et al., 2013](#); [McNally et al., 1993](#); [Rampazzo et al., 2014](#)).

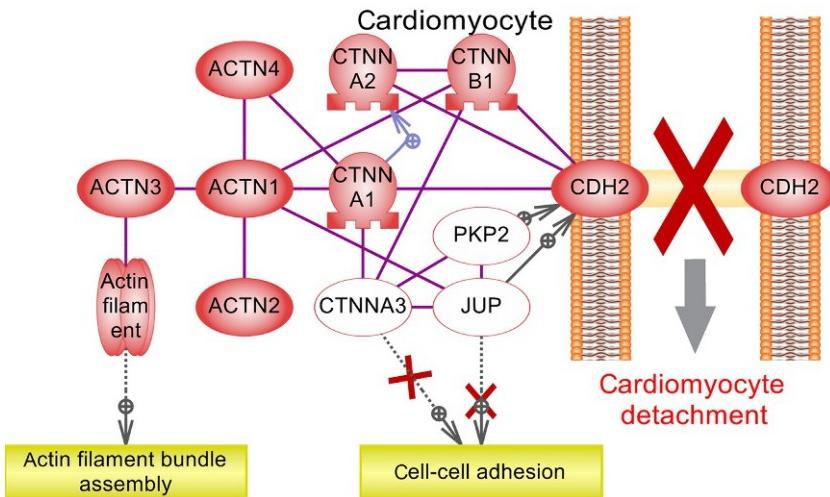


FIG. 13 Pathway 1: Adherens junction dysfunction in cardiomyocytes in ARVD.

## Pathway 2

### Desmosome dysfunction in cardiomyocytes in ARVD (Fig. 14)

#### Incoming signals

Desmosomes are major cell-cell junctions that are particularly abundant in epidermal cells and cardiomyocytes. ARVD may be caused by genetic defects of the structure of myocardial desmosomes. Desmosomes are multiprotein complexes that form intercellular contacts on the surface of heart muscle cells. Mutations in many proteins that comprise desmosomes can have harmful effects on cardiomyocyte function.

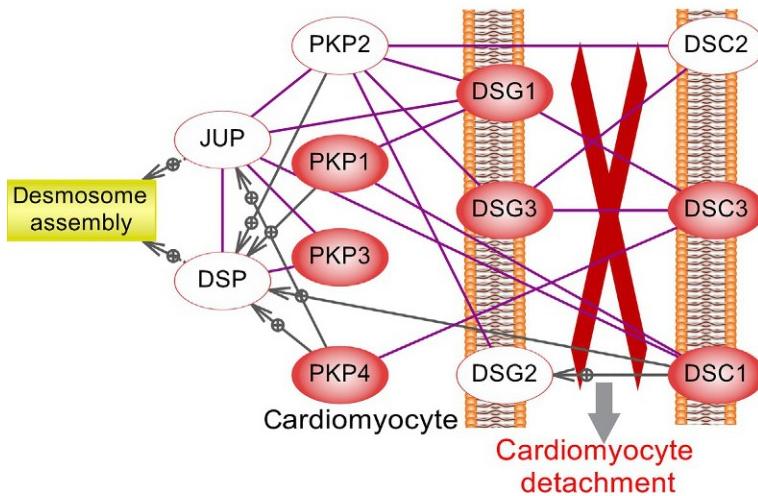
#### Outcome effects

Defective desmosomes cannot sustain the constant mechanical stress inherent in contracting cardiomyocytes, resulting in cardiomyocyte detachment and the consequent impairment of mechanical and electrical cell junctions.

#### Signaling

JUP is a cytoplasmic protein found in both submembranous plaques of desmosomes and in intermediate junctions. This protein, a member of the catenin family, forms distinct complexes with cadherins. It contains a repeating amino acid motif called the armadillo repeat. Mutations of *JUP* affect the structure and distribution of mechanical and electrical cell junctions. The desmoplakin (DSP) protein, along with JUP, anchors desmosomal cadherins by forming an ordered array of nontransmembrane proteins, which then bind to keratin intermediate filaments. Abnormalities in DSP lead to the instability of desmosome.

Abnormalities in plakophilin 2 (PKP2), the desmogleins and desmocollins are also thought to perturb intercellular connections and lead to cardiac arrhythmia. Desmoglein-2 (DSG2) is an essential component of desmosomes in the myocardium. Desmocollins (DSC2) bind desmogleins through their extracellular domains in a  $\text{Ca}^{2+}$ -dependent manner. Their cytoplasmic domains have binding sites for PKP2. Only one of three desmocollin-2 (DSC2) isoforms is present in the cardiac tissue. A mutated isoform of DSC2 is unable to bind PKP2 that may result in desmosome dysfunction (Campuzano et al., 2013; McNally et al., 1993; Rampazzo et al., 2014).



**FIG. 14** Pathway 2: Desmosome dysfunction in cardiomyocytes in ARVD.

## Pathway 3

### Cardiomyocyte dysfunction unrelated to cell junctions in ARVD ([Fig. 15](#))

#### Incoming signals

Although the majority of ARVD-causing mutations are in genes encoding desmosomal proteins, a few ARVD mutations have been detected in genes unrelated to intercellular junction complexes. These include ryanodine receptor 2 (*RYR2*), transforming growth factor beta 3 (*TGFB3*), transmembrane protein 43 (*TMEM43*), desmin (*DES*), titin (*TTN*), lamin A/C (*LMNA*), and sodium voltage-gated channel alpha subunit 5 (*SCN5A*).

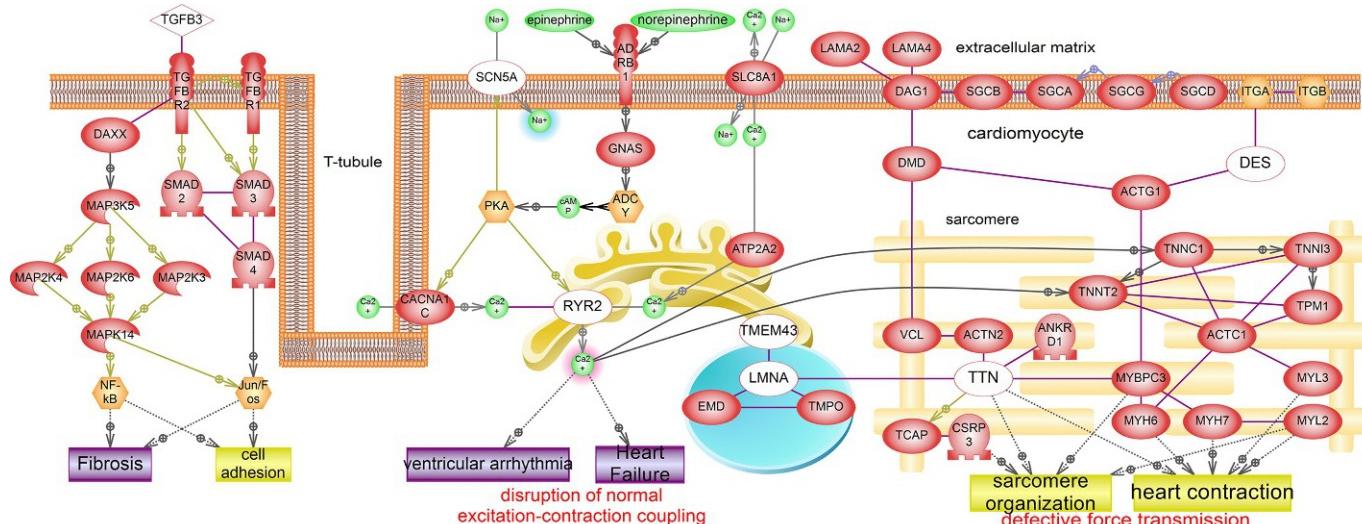
#### Outcome effects

Mutated proteins cause cardiomyocyte dysfunction due to defective force transmission between cells, the disruption of normal excitation-contraction coupling, and impairment of cardiomyocyte adhesion and fibrosis induction.

#### Signaling

Mutations in the *RYR2* gene have been shown to account for an atypical form of ARVD associated with polymorphic ventricular arrhythmias and for catecholaminergic polymorphic ventricular tachycardia, a peculiar malignant arrhythmic disease. Mutations in *RYR2* are thought to result in an uncontrolled calcium leak into the cardiac myocyte, leading to arrhythmia. *TGFB3* regulates cytokine-stimulating fibrosis and modulates cell adhesion. *TMEM43* encodes a novel transmembrane protein, which may be a target of PPARG. The precise functions of *TGFB3* and *TMEM43* in ARVD are still poorly known ([Campuzano et al., 2013](#); [McNally et al., 1993](#); [Rampazzo et al., 2014](#)).

## II. Human disease pathways

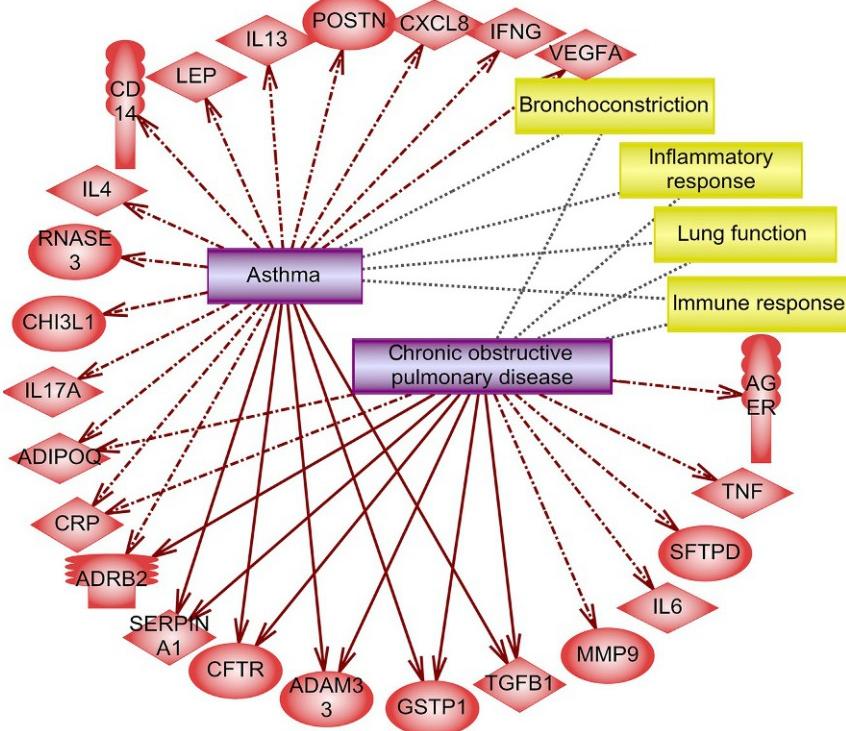


**FIG. 15** Pathway 3: Cardiomyocyte dysfunction unrelated to cell junctions in ARVD.

## References

- Disease numbers # 107970 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code I42.8. Diseases of the circulatory system (I00-I99). (ICD-10, <https://icdlist.com>). ICD-11: disease code BC43.6.
- Campuzano, O., Alcalde, M., Allegue, C., Iglesias, A., García-Pavía, P., Partemi, S., Oliva, A., Pascali, V.L., Berne, P., Sarquella-Brugada, G., Brugada, J., Brugada, P., Brugada, R., 2013. Genetics of arrhythmogenic right ventricular cardiomyopathy. *J. Med. Genet.* 50, 280–289. <https://doi.org/10.1136/jmedgenet-2013-101523>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- McNally, E., MacLeod, H., Dellefave-Castillo, L., 1993. Arrhythmogenic right ventricular cardiomyopathy. In: Adam, M.P., Ardinger, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J., Mefford, H.C., Stephens, K., Amemiya, A., Ledbetter, N. (Eds.), GeneReviews®. University of Washington, Seattle, Seattle, WA.
- Rampazzo, A., Calore, M., van Hengel, J., van Roy, F., 2014. Intercalated discs and arrhythmogenic cardiomyopathy. *Circ. Cardiovasc. Genet.* 7, 930–940. <https://doi.org/10.1161/CIRCGENETICS.114.000645>.

# Diseases of the respiratory system



## O U T L I N E

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Chronic obstructive pulmonary disease	425

Respiratory diseases develop due to disturbances of the respiratory tract organs including the trachea, bronchi, bronchioles, alveoli, pleura and pleural cavity, nerves, and muscles of breathing and are frequent in humans. Our modern lifestyle and low air quality provoke disorders of the respiratory system, which are often chronic. This chapter focuses on two major chronic respiratory illnesses: asthma and chronic obstructive pulmonary disease (COPD). Cystic fibrosis, as an example of a chronic and monogenic respiratory disease, is described in the chapter on endocrinological diseases in accordance with the World Health Organization classification (<https://www.who.int/classifications/icd>).

Both asthma and COPD are chronic inflammatory lung diseases that cause obstructed airflow from the lungs.

Asthma and COPD are striking examples of how complex interactions between genetic variations and environmental triggers can cause changes in cell signaling and start allergic, immunological, and inflammatory responses. Asthma is triggered by exposure to air pollution and allergens; other potential triggers include medications. The frequency of asthma has increased over the last few decades due to increased air pollution.

COPD is an inflammatory lung disease, which is hardly treatable, with tobacco smoking recognized as the most common cause. COPD refers to a group of diseases. Chronic bronchitis and emphysema are older terms now used for different types of COPD. These terms are no longer included in the formal definition of COPD although they are still used clinically.

The key symptoms of asthma and COPD, cough, shortness of breath, and sputum production, are similar, yet the mechanisms underlying the pathogenesis of asthma and COPD are not the same. Hyperstimulation of the immune response leads to asthma progression, while the inflammation-mediated destruction of the pulmonary tissues is a critical element in the development of COPD.

## CHAPTER

## 9.1

## Asthma

Asthma is a breathing disorder characterized by inflammation of the airways and recurrent episodes of asthma attacks (breathing difficulty), which are triggered by bronchial hyperresponsiveness to the irritation.

The National Asthma Education and Prevention Program (NAEPP) guidelines define asthma as “a chronic inflammatory disease of the airways in which many cells and cellular elements play a role: in particular mast cells, neutrophils, eosinophils, T lymphocytes, macrophages, and epithelial cells.” In susceptible individuals, this inflammation causes recurrent episodes of coughing (particularly at night or early in the morning), wheezing, breathlessness, and chest tightness. (*Ferri and Ferri, 2018*).

In the asthma attack, both bronchoconstriction and immune reaction occur. Muscles around the airways contract and narrow that makes breathing difficult; also, mucus overproduction leads to the shortness of breath and coughing. During status asthmaticus (severe acute asthma) shortness of breath may persist for several hours and does not respond to standard therapy such as inhaled beta-agonists or subcutaneous epinephrine (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Allergic asthma is the most common form of the disorder, and sometimes, it is only part of a series of allergic conditions, which a patient has. This state is referred to as “the atopic march.” Various irritants such as inhaled allergens, respiratory infections, tobacco smoke, cold air, or physical activity can trigger allergic asthma.

Asthma can progress at any age though symptoms of allergic or extrinsic asthma develop in 50%–80% of children before 5 years of age. Nonallergic or intrinsic asthma manifests in adulthood, typically in response to respiratory tract infections, drugs, or stress (*Ferri and Ferri, 2018*).

Some forms of asthma no doubt result from a genetic predisposition, but their inheritance pattern is unknown. There are more than 100 mutated genes associated with allergic asthma with different groups identified in different populations. However, in general, asthma is considered to be a complex condition caused by genetic and environmental factors and lifestyle. As with other complex diseases, every patient with asthma

has their own pattern of foundations that manifest themselves in a typical clinical picture (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Many patients with allergic asthma have an immunological reaction that is driven predominantly by type 2 T-helper cells (Th2 cells), but not Th1 cells. Th2 cell reactions are associated with the production of immunoglobulin E (IgE) by B cells, activation of eosinophils, and the involvement of other cells and mediators that enhance the characteristic bronchial hyperresponsiveness:

**Pathway 1. Th2 cell response in asthma (Fig. 1).**

Eosinophilic and/or neutrophilic inflammation is an important sign of allergic asthma. In asthma, eosinophils are attracted to the lungs by cytokines released by Th2 cells. In the lungs, eosinophils release the content of their granules, which leads to cellular damage. On the other hand, airway neutrophilia is one of the hallmarks of severe asthma:

**Pathway 2. Eosinophilia and neutrophilia in asthma.**

Eosinophil migration and activation (Fig. 2).

Eosinophil apoptosis (Fig. 3).

Neutrophil chemotaxis and activation (Fig. 4).

Mucus overproduction is a central feature of bronchial asthma, contributing to mortality seen in the disease. Goblet cells are the primary mucus-producing cells. In asthma the airway surface liquid (ASL) thickness increases due to the overflow of water and the secretion of adds to excessive mucin, which accumulations in the lungs with asthma:

**Pathway 3. Airway surface liquid (ASL) thickness and mucus accumulation in asthma.**

Production and release of mucins, mucus metaplasia (Fig. 5).

Regulation of airway surface liquid (ASL) thickness (Fig. 6).

Eosinophils, mast cells, and basophils release histamine and other mediators that induce excessive narrowing of the airways and to bronchoconstriction. Excessive shortening (contraction) of airway smooth muscle (ASM) cells and airway tissue remodeling develops as the disease progress is responsible for breathing difficulty. Eventually, the muscles around the airways may enlarge through hypertrophy, further narrowing the airways:

**Pathway 4. Airway smooth muscle cells and airway remodeling in asthma.**

Airway smooth muscle cells contraction (Fig. 7).

ASM proliferation and airway tissue remodeling (Fig. 8).

## Key cellular contributors and processes

Antigen-presenting cells

Cell

Antigen-presenting cells (APCs) are a large group of various cells that trigger the cellular immune response by processing an antigen and exposing it in a form recognizable by T cells in the process known as antigen presentation.

Chemotaxis

Process

Chemotaxis is directional movement or orientation of cells along a gradient of concentration of a chemical substance.

Eicosanoids

Process

Eicosanoids are lipid signaling mediators derived from arachidonic acid and related polyunsaturated fatty acids.

Hyperplasia

Process

Hyperplasia is a rise in the number of cells in an organ or tissue due to their increased proliferation. Hyperplasia often precedes the development of cancer.

Hypertrophy

Process

Hypertrophy is an increase in the size of an organ or tissue caused by an increase in the size of their cells.

Major histocompatibility complex class II

Protein or gene

The major histocompatibility complex (MHC) class II is a heterodimeric protein complex on the surface of antigen-presenting cells. The MHC class II molecules have a fundamental role in processing extracellular antigens and presenting them to T cells.

Mucus

Process

Mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may tether together at the fluid surface by oligomeric mucin protein complexes.

## Naïve T cells

### Cell

Naïve T cells are mature T cells in the bone marrow that have gone through the process of positive and negative selection and can respond to newly recognized pathogens.

## Phagosome (phagocytosis)

### Anatomic structure

Phagocytosis is a form of endocytosis by which a cell internalizes large ( $>0.5\text{ }\mu\text{m}$ ) particles via the formation of an internal compartment known as phagosome, which is further fused with a lysosome for degradation. Professional immune cells (macrophages, neutrophils, and others) employ phagocytosis to remove invading pathogens.

## Pathway 1

### Th2 cell response in asthma (Fig. 1)

#### Incoming signals

Traditionally, asthma has been known as a T-helper (Th2) cell-mediated disease. T-helper cells are lymphocytes that release cytokines and present antigen-MHC complexes on their surface. Th2 cells are responsible for many types of downstream allergic events in asthma that include activation of B cells, production of IgE antibodies, and eosinophilic inflammation. In healthy individuals, IgE is produced in response to parasitic worms (helminths). In individuals with a sensitizing predisposition, IgE is produced after contact with particular antigens (Yang et al., 2014).

Antigens, which induce an immune response, are called immunogens, and if they elicit allergies, they are called allergens. There are now 137 inhaled allergen families according to the AllFam (<http://www.meduniwien.ac.at/allfam/>). Pathogens and molecules derived from a pathogen such as endotoxin lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, also increase the risk of asthma.

Antigen-presenting cells (APCs) take up various foreign molecules, process them, and present components of them on their cell surface as antigen-MHC complexes. Cells loaded with antigens migrate to lymph nodes where they induce differentiation of naïve T cells toward either the Th1 or Th2 cell lineages. Allergens activate signaling pathways in antigen-presenting cells and in the airway epithelium. Activated cells start to express and release various cytokines, chemokines, and costimulatory molecules, which in turn attract immune cells and promote their differentiation (Hall and Agrawal, 2014; Kaiko et al., 2008).

#### Outputs

T-helper 2 (Th2) cells produce cytokines (principally IL-5 and IL-13) that are crucial for the development of asthma. IL-5 stimulates the differentiation of eosinophils; IL-4 and IL-13 stimulate contraction of smooth muscle whereas together; and with IgE, they can stimulate the production of mucus production (Erle and Sheppard, 2014; Hall and Agrawal, 2014).

Activated Th2 cells stimulate IgE production, which forms a complex with allergens and binds to receptors on the surface of eosinophils or mast cells resulting in degranulation of those cells and the consequent obstruction of airways.

## Signaling

### ***Antigen-presenting cells in asthma***

The antigen-presenting cells (dendritic cells, macrophages, and lung epithelial cells) are the first cells to recognize antigens. The antigens/allergens are also recognized through antigen-presenting cell surface pattern recognition receptors (PPRs), including the toll-like receptors (TLRs), C-type lectins (such as CLEC7A or mannose receptor, C type 1 (MRC1)), NOD-like receptors, and they are also absorbed during phagocytosis (Hadebe et al., 2018; Hall and Agrawal, 2016).

Individual characteristics of allergens such as their protease activity, surface features, and glycosylation patterns (predominantly mannosylation) determine whether or not PPRs distinguish them from other nonallergenic proteins. For example, C-type lectins in asthmatic dendritic cells recognize various exogenous ligands through the presence of mannose on the surface of pathogens (Al-Ghouleh et al., 2012; Loke et al., 2016).

MRC1 is the most studied C-type lectin receptor, and it plays a special role in asthma. MCR1 takes up allergens derived from house dust mites (HDM) including the peptidase 1 precursor (DerP-1) and binds to several other allergens (including major dog allergen (Can f 1), cat allergen (Fel d 1), and the major peanut allergen (Ara h 1)). Expression of MRC1 was observed to be elevated in asthmatic dendritic cells (Hadebe et al., 2018).

MCR1 also directly promotes the phagocytosis of allergens by macrophages through their interaction with the FcR gamma chain. FcR gamma recruits GRB2, which is involved in the activation of the complex RAC1/CDC42/PAK1. The formation of this complex supports phagocytosis. During MC1R-mediated phagocytosis the actin cytoskeleton is mobilized around nascent phagosomes (Rajaram et al., 2017). Phagocytosis is a complex, gradual, and stepwise process. Therefore only the simplified model of MC1R-mediated phagocytosis is shown on the pathway.

MC1R signaling leads to the transcriptional activation of several cytokines in addition to the phagocytic signals. IL-1, IL-6, CSF2, TNF, and IL-12 are possibly produced by MC1R signaling. However, the specific mechanisms of the cascade downstream of MC1R activation are unknown.

Nuclear factor kappaB (NF- $\kappa$ B) transcription factors are crucial elements in the PPR signaling, leading to the synthesis of different proteins by antigen-presenting cells. PPRs stimulate the release of chemokines (CCL17 and CCL22 and CCR7), cytokines (CSF2, IL-2, IL-4, IL-6, TNF, TSLP, etc.), and other mediators, which in turn induce the Th2 cell immune response, mucous hyperplasia, and smooth muscle contraction (Erle and Sheppard, 2014).

Allergens (such as house dust mite allergens) were shown to induce the expression of CCR7 and CSF2, which can themselves attract dendritic cells to lymph nodes (Fyhrquist-Vanni et al., 2007).

Patients with asthma have a high level of cytokine thymic stromal lymphopoietin (TSLP), which is produced mainly by epithelial cells and fibroblasts. TSLP regulates the survival of Th2 cells and several other cell types in asthma (including B cells, dendritic cells, eosinophils, basophils, mast cells, and smooth muscle cells) (West et al., 2012) (also see Asthma: Pathway 4).

The inflammasome, NOD-like receptor sensor pathogens, and damage-associated molecular patterns (DAMP) promote the synthesis and activation of proinflammatory interleukins IL-33, IL-18, and IL-1B by caspase 1 (CASP1) (Davis et al., 2011).

Patients with asthma show high levels of IL-33 in both their serum and their lungs. It is assumed that IL-33 is expressed mainly in nonhematopoietic cells such as epithelial cells or it is released from necrotic cells. IL-33 plays a wide range of roles in the context of allergic airway inflammation (Besnard et al., 2011).

IL-33 released by APCs displays feedback effects on APC function by enhancing activation of the NF- $\kappa$ B and MAPK pathways. IL-33 signaling also enhances the expression of CCR3 in dendritic cells, macrophages, and mast cells, which are involved in the recruitment of eosinophils to the inflamed lung in asthma. IL-33 also triggers the production of IL-13 (and perhaps CCL17) from eosinophils, dendritic cells, and mast cells, thus reinforcing the accumulation of lymphocytes in the airways (Milovanovic et al., 2012; Nechama et al., 2018; Stolarski et al., 2010) (also see Asthma: Pathway 2. Eosinophils apoptosis).

### **T-helper cell activation**

Mucosal dendritic cells loaded with antigens migrate from the lungs to the T-cell area of mediastinal lymph nodes (MLNs). The cooperative interplay of antigens with cytokines in asthma induces clonal expansion of antigen-specific Th2 cells. IL-4 is the major cytokine in the Th2 lineage. The transcription factor STAT6 drives Th2 differentiation, in part, by enhancing the expression of GATA3, which in turn induces the secretion of IL-5 and IL-13 by Th2 cells (Kaiko et al., 2008) (for T-helper cell activation, see Chapter 13: Overview of Diseases of the Immune System). Many other cytokines, including TSLP, GSF2, IL-1, and IL-33, stimulate the secretion of Th2 cytokines (Erle and Sheppard, 2014).

Increased levels of Th17 cells and high levels of IL-17A are correlated with severe asthma. Th17 cells are a subset of T-helper cells, which attract and activate neutrophils (Lindén and Dahlén, 2014) (see Asthma: Pathway 2. Neutrophils chemotaxis and activation).

### **IgE antibodies production**

Th2 cells deliver antigens complexed to MHC II to B-cell receptors (BCR, CD79A/IgM/CD79B) on immature B cells. Cytokines secreted by T cells provide the second signal for B-cell proliferation and their

differentiation into plasma cells. During B-cell maturation, genetic recombination and isotype switching of immunoglobulin genes occur (not shown). Isotype switching is necessary to start the transcription of immunoglobulins. Although some cytokines are involved in promoting B cells to produce antibodies and generate plasma cells, only IL-4 and IL-13 are known to promote immunoglobulin isotype switching to IgE ([Matsushita and Yoshimoto, 2014](#)).

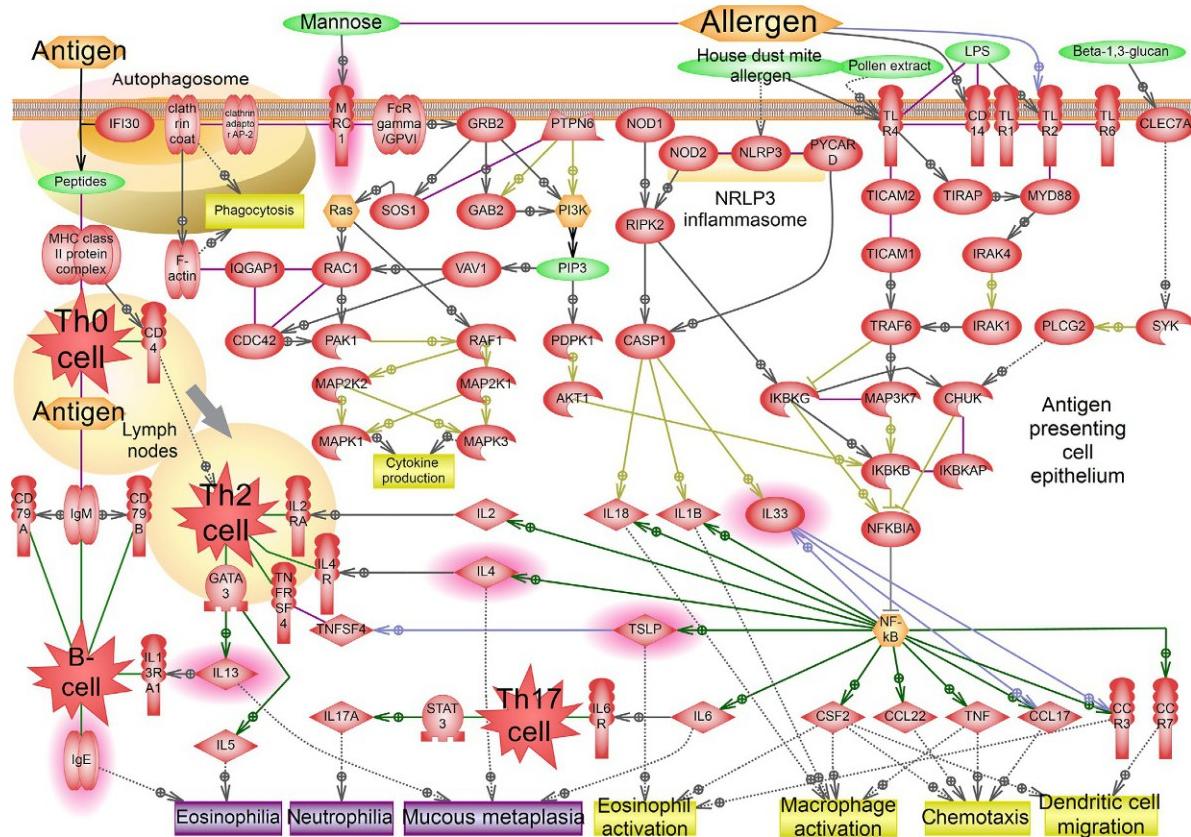


FIG. 1 Pathway 1: Th2 cell response in asthma.

## Pathway 2

### Eosinophilia and neutrophilia in asthma

#### Incoming signals

Eosinophilic and/or neutrophilic inflammation is essential signs of allergic asthma. In healthy humans, eosinophils comprise 1%–6% of all white blood cells and circulate in the blood for several hours. In the lungs, eosinophils appear only in the case of disease, such as asthma, and they survive up to 12 days afterward. Airway neutrophilia is one of the hallmarks of severe asthma. Neutrophils, the largest class of white blood cells, are major cellular antibacterial agents in inflammation since they combine the functions of granulocytes and phagocytes. Neutrophilia in asthma is associated with severe asthma and persistent steroid resistance. Th2 cell-related cytokines drive eosinophilic inflammation, while the Th17 cell response is associated with infiltration of neutrophils (Pelaia et al., 2015).

The translocation of eosinophils from bone marrow to the airways is caused by the binding of ligands known as exotoxins (including chemokines such as C-C motif chemokine ligands CCL11 CCL24 and CCL5, CCL26) to CCR3. These exotoxins are mainly produced by epithelial and endothelial cells (Isgrò et al., 2013; Provost et al., 2013). Levels of CCL24 and CCL26 were increased in airway epithelial brushings from patients with asthma (Coleman et al., 2012). The additional chemoattractants and activators of eosinophils in inflammation include leukotriene B4 (LTB4), platelet-activating factor, and prostaglandin D2 (PGD2). IL-5, IL-13, and colony-stimulating factor 2 (CSF2) are main cytokines that prolong eosinophil survival.

IL-5 serum levels are elevated in asthma (Joseph et al., 2004). High levels of circulating IL-25, IL-33, IL-33, and brain-derived neurotrophic factor (BDNF) have also been documented in acute allergic asthmatics. Other cytokines, chemokines, small molecules, and inflammatory mediators can also activate eosinophils but probably to a lesser extent (Shen and Malter, 2015).

#### Outcome effects

The effector functions of eosinophils and neutrophils are related to their cytotoxic effect. Upon activation, eosinophils release the content of their granules at the site of inflammation. The eosinophil-specific enzymes proteoglycan 2 (PRG2), ribonuclease A family member 3 (RNASE3), eosinophil peroxidase (EPX), and ribonuclease A family member 2 (RNASE2) are directly implicated in cell damage. Other mediators derived from eosinophils are major contributors to local inflammation in airways, mucus hypersecretion, and tissue fibrosis. Eosinophils can play either proallergic or defensive antiallergic roles. For example, eosinophils are capable of

both the release of histamine and its neutralization, either enzymatically or through phagocytosis.

Neutrophils absorb pathogens tagged with opsonins, which are molecules that bind the cell surface and facilitate phagocytosis. Upon degranulation (exocytosis), neutrophils release the content of their granules, proteins, and ROS, and other small molecules, which possess antibacterial properties, induce the phagocytosis of pathogens and dying cells and slow down the inflammatory reaction. In the context of asthma, excess neutrophils and eosinophils lead to persistent bronchoconstriction, mucus secretion, and inflammation.

Eosinophils and neutrophils do not synthesize and release all mediators immediately, but instead, they do so selectively. Mechanisms that control the selective synthesis and release of proinflammatory mediators require further research.

In the absence of prosurvival factors, neutrophils, eosinophils, and basophils die by intrinsic apoptosis within a short time after maturation. The apoptosis and clearance of eosinophils by macrophages are attenuated in asthma.

## Signaling

### ***Eosinophil migration and activation (Fig. 2)***

CCR3 receptor signaling stimulates the migration, calcium-dependent degranulation, and activation of eosinophils via a G protein-dependent mechanism (Houimel and Mazzucchelli, 2013; Provost et al., 2013). Degranulation and chemotaxis require profound rearrangements of the actin cytoskeleton. The precise mechanism of signaling that leads to remodeling of the cytoskeleton in eosinophils is unknown. Calcium influx is a key signaling event or at least a marker in eosinophil degranulation. The Rho family of small GTP-binding proteins (RhoA) and their effector kinases ROCKs and MAPK1/MAPK3 were activated downstream of CCR3 signaling and participated in eosinophil chemotaxis (Adachi et al., 2001).

The first interaction of circulating eosinophils occurs with cell adhesion molecules, which are expressed on the inflamed vascular endothelium of blood vessels (such as vascular cell adhesion molecule 1, VCAM1) and eosinophils (such as integrin alpha M, ITGAM). Cell adhesion molecules are also involved in the remodeling of the cytoskeleton in eosinophils (Barthel et al., 2008).

Like other granulocytes, eosinophils are activated by a signal from IgE. Polymorphisms within the gene encoding Fc fragment of IgE receptor II (FCER2), also known as CD23 receptor, leading to low affinity for IgE, have been associated with an increased risk of exacerbations in patients with asthma and high serum IgE levels (Chan et al., 2014).

### Eosinophil survival (Fig. 3)

The Th2 cell cytokine IL-5 plays a dominant role in promoting the local survival of eosinophils in the airways. IL-5 activates the LYN/SYK and JAK2 pathways promoting eosinophil survival and proliferation (Adachi and Alam, 1998). IL-5 may trigger the differentiation of eosinophils through its interaction with syntenin and activation of the transcription factor SRY (sex-determining region Y)-box 4 (SOX4) (Beekman et al., 2009). CSF2 was also shown to be a critical factor in regulating the fate of eosinophils. CSF2 shares with IL-5 and IL-3 a common beta chain, termed the cytokine-receptor homology module, in their receptors, which is a member of the class I cytokine receptor superfamily, while the alpha subunit remains unique (Murphy and Young, 2006).

In addition, IL-5 and CSF2 appear to have an influential role in inhibiting eosinophil apoptosis, for example, by inducing the expression of the serine/threonine protein kinase PIM1 (Pim-1 proto-oncogene, serine/threonine kinase) (Shen and Malter, 2015).

Levels of IL-25 secreted by epithelial cells and expression of its receptors (IL-17RA/IL-17RB, interleukin-17 receptor A and B) on eosinophils were increased in allergic asthma patients. Details of the downstream signaling pathways of IL-25 in eosinophils is still unclear but induction of the MAPK and NF- $\kappa$ B pathways along with expression of chemokines (CCL2 and CCL3L3) and interleukins (IL-8 and IL-6) were detected after IL-25 binding (Tang et al., 2018; Wong et al., 2005).

BDNF derived from the airway epithelium in response to an allergic inflammatory reaction has been described as a modulator of airway hyperresponsiveness in the allergic reaction in asthma (Hahn et al., 2006).

IL-33 derived from dying cells or produced by mucosal cells binds to its plasma membrane receptor on eosinophils and other cells to induce the NF- $\kappa$ B mediated expression of inflammatory mediators. For example, activation of the IL-33 signaling pathway enhances the expression of CCR3 in eosinophils, thereby promoting elevated eosinophil numbers and increased mucous secretion. IL-33 can trigger the production of IL-13 or CCL17 by eosinophils, dendritic cells, and mast cells, and it is a part of other processes in asthma-related pathology (Milovanovic et al., 2012; Stolarski et al., 2010).

All of the above mediators may induce exocytosis and degranulation in eosinophils by different signaling pathways. An increase in  $\text{Ca}^{2+}$ , which leads to the synthesis of arachidonic acid and lysophospholipid, is a crucial mechanism that supports the fusion of granules with membranes and the release of their contents.

### Eosinophil apoptosis (Fig. 4)

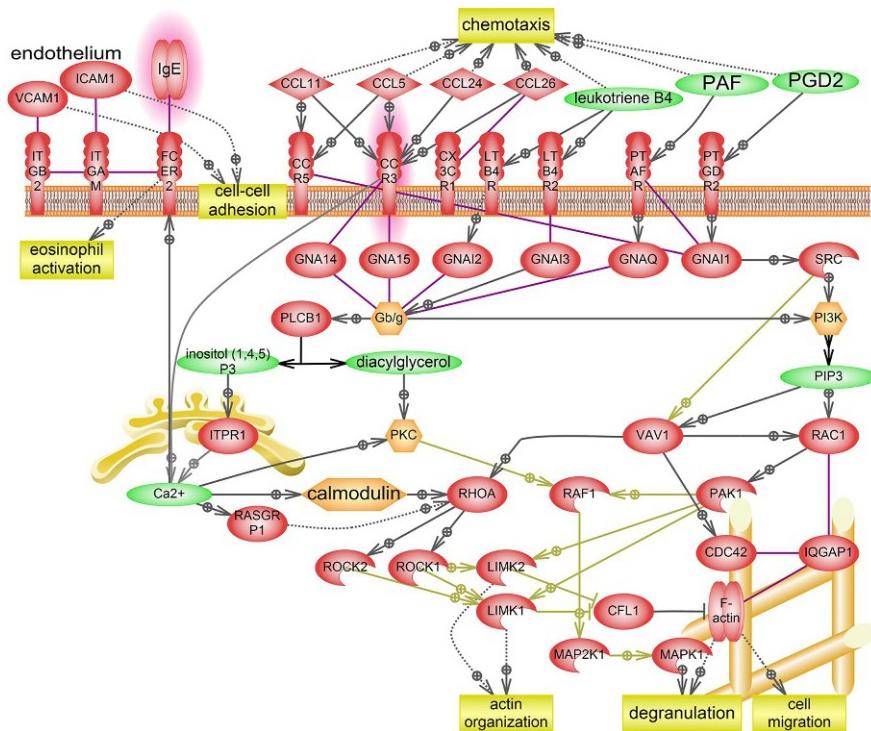
TGFB1 and other ligands bind to the death receptor FAS to stimulate eosinophil apoptosis and apoptosis of other cells (not shown).

Sialic acid-binding Ig-like lectin 8 (SIGLEC8) is an important target for asthma-related drug target research as it is selectively expressed on

human eosinophils at high levels and it mediates eosinophil apoptosis. Also a significant association between single-nucleotide variants in the SIGLEC8 gene sequence and asthma was observed among African American, Japanese, and Brazilian populations. SIGLEC8-induced eosinophil death was enhanced by activation of the IL-5 and CSF2 pathways. The exact mechanism for SIGLEC8 signaling in eosinophils remains unknown. SIGLEC8 binds to sialic acids in glycoproteins and glycolipids on lung epithelium and probably activate caspase8/caspase3 and mitochondrial apoptosis (Feng and Mao, 2012; Shen and Malter, 2015).

### **Neutrophil chemotaxis and activation (*Fig. 4*)**

Neutrophilic asthma is driven by the chemokine CXCL8 (IL-8), which is released by airway epithelium and other tissues during inflammation (Rahman et al., 2014). IL-17F was shown to induce IL-8 expression in vitro and to promote neurotrophic activation in mouse models of asthma. IL-17F is expressed in cell types involved in allergic airway inflammation including activated Th17 cells, basophils, mast cells, and probably in eosinophils. Asthmatic patients have significantly higher serum levels of IL-17F compared with healthy subjects (Ota et al., 2014; Pelaia et al., 2015).



**FIG. 2** Pathway 2: Eosinophilia and neutrophilia in asthma. Eosinophil migration and activation.

## II. Human disease pathways

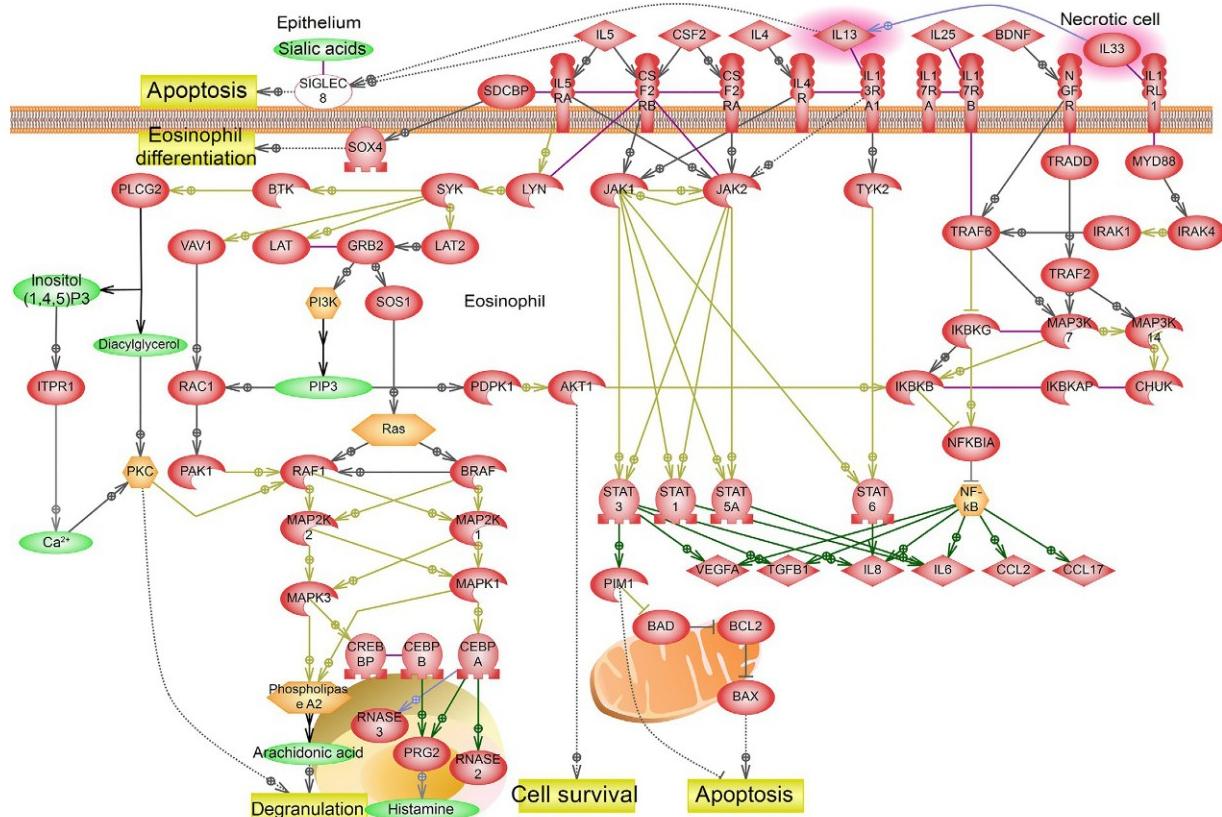
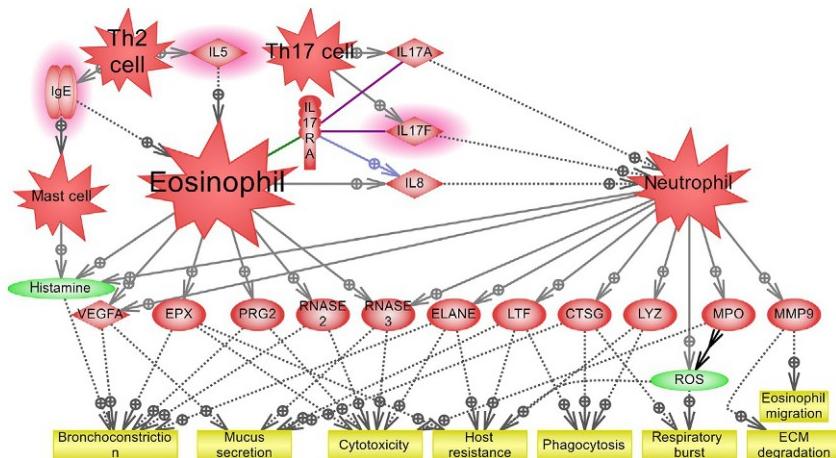


FIG. 3 Pathway 2: Eosinophilia and neutrophilia in asthma. Eosinophil apoptosis.



**FIG. 4** Pathway 2: *Eosinophilia and neutrophilia in asthma*. Neutrophil chemotaxis and activation.

## Pathway 3

### Airway surface liquid thickness and mucus accumulation in asthma

#### Incoming signals

Mucus hypersecretion is one of the main features of several hypersecretory respiratory diseases including chronic obstructive pulmonary disease (COPD), cystic fibrosis, and asthma. In asthma, mucus forms plugs that are difficult to dislodge from the airways (Rogers, 2004).

Airway mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may tether together at the fluid surface by oligomeric mucin protein complexes. The most abundant gel-forming mucins in the human tracheobronchial epithelium are mucin 5AC (MUC5AC), produced by surface epithelial mucus-secreting cells (mainly by goblet cells), and mucin 5B (MUC5B), produced by submucosal glands (Bonser and Erle, 2017).

TNF and other cytokines released by Th2 cells and epidermal growth factor receptor (EGFR) signaling pathway induce goblet cell hyperplasia and the expression of mucins. Activation of TLRs by pathogens also stimulates mucin synthesis.

The increase of goblet cell numbers is a common feature observed in both asthma and COPD. Hyperplasia of goblet cells or increased levels of goblet cell differentiation from club cells (mucus metaplasia) both may lead to mucus overproduction.

A well-balanced airway surface liquid (ASL) system is required for normal mucus clearance. In asthma, fluids overflow into the lumen increasing ASL thickness leading to airway dysfunction.

#### Outcome effects

Mucins secreted by goblet cells contribute heavily to the viscoelastic properties of the extracellular mucous layer. Mechanical clearance of mucus (mucociliary clearance) is a primary defense mechanism of the airways against inhaled microorganisms, but it is complicated in asthma due to excessive mucin production and mucus plug formation. Accumulation of mucus contributes to the failure of phagocytes and dendritic cells; it leads to airway obstruction and respiratory infections in patients with asthma. Inflammation also increases expression  $\text{Cl}^-$  ion channels resulting in increased hydration and thickness of airway surface liquid, which together exacerbate the clinical features of asthma.

## Signaling

### **Mucin production (Fig. 5)**

Our understanding of the regulation of mucin production in patients with asthma, based mainly on animal model studies, remains incomplete.

The Th2-type interleukins (IL-13, IL-6, IL-4, and IL-9) released by activated T cells, mast cells, and basophils bind their receptors on goblet cells to induce mucin expression and goblet cell proliferation. The proinflammatory cytokines TNF and IL-1B, as well as pathogens, activate TLR-related signaling to induce transcription of mucin genes. Growth factors such as epidermal growth factor (EGF) and transforming growth factor alpha (TGFA) trigger epidermal growth factor receptor (EGFR) signaling, which also induces mucin synthesis and secretion (Lai and Rogers, 2010; Williams et al., 2006; Wu et al., 2017).

The listed signaling pathways activate several well-known transcription factors, which regulate expression of mucin (MUC5AC and MUC5B) genes. IL-13 triggers mucin transcription by activating STAT6 and inhibiting forkhead box protein A2 (FOXA2). It also activates IL-1B; TNFA, via NF-kb; and hypoxia-inducible factor 1-alpha (HIF1A) (Wu et al., 2017). EGFR signaling is involved in the activation of the JUN, FOS, and SP1 transcription factors and leads to mucin synthesis (Burgel and Nadel, 2008; Perrais et al., 2002).

Prostaglandin E2, a metabolite of arachidonic acid, levels are increased in patients with asthma, and it can participate in the elevation of mucin production in goblet cells. Prostaglandin E2 activates G protein receptor signaling, which in turn activates the transcription factor CAMP responsive element binding protein 1 (CREB1) (Akaba et al., 2018).

There is the hypothesis that IL-13 and IL-4 signaling can enhance MUC5AC expression by activating the enzyme arachidonate 15-lipoxygenase (ALOX15), which in turn oxidizes unsaturated fatty acids. The product of ALOX15, 15HETE-PE can interact with phosphatidylethanolamine binding protein 1 (PEBP1) to activate the RAF1/MAPK pathway and amplify IL-4R downstream pathways. ALOX15 and PEBP1 interactions occur primarily at the cell membrane (Zhao et al., 2011). Epithelial ALOX15 expression increases in patients with asthma (Zhao et al., 2009).

### **Mucins release (Fig. 5)**

Myristoylated alanine-rich C-kinase substrate (MARCKS) has been shown to be a major regulator of airway mucin-containing granule exocytosis, but the precise mechanism of its regulation is unknown. Under normal conditions, intracellular MARCKS is attached to the plasma membrane. When MARCKS is phosphorylated by PKC, it translocates from the plasma membrane to the cytoplasm. Vesicle-associated membrane protein

8 (VAMP8), synaptotagmin 2 (SYT2), and other vesicle-associated proteins are also essential for the regulating mucin granule exocytosis in goblet cells (Fang et al., 2013; Jones et al., 2012; Raiford et al., 2011).

### **Mucus metaplasia (Fig. 5)**

There is some evidence to support the notion that club cells are the main progenitors for goblet cells and that they increase goblet cell numbers after contact with an allergen in asthma. Our understanding of the molecular mechanisms underlying club cell differentiation originate from studies of murine models, yet it remains poorly developed (Bonser and Erle, 2017; Volckaert and De Langhe, 2014). Club cells (Clara cells) are a type of bronchiolar epithelial cell, which secretes the secretoglobin family 1A member 1 (SCGB1A1) protein to protect the bronchiole lining. The club cell's own mucin expression is explicitly limited to a subset of club cells (generations 2–4) that continue to express SCGB1A1. In mice, club cells release IL-13 via STAT6 and NOTCH2 signaling and can induce goblet cell metaplasia. Transcription factors such as SAM pointed domain-containing ETS transcription factor (SPDEF) and hepatocyte nuclear factor 3-gamma (FOXA3) are required for goblet cell differentiation induced by allergens and by IL-13 exposure. They regulate the expression of a group of genes associated with mucin biosynthesis and secretion (Chen et al., 2009; García et al., 2014; Reader et al., 2003; Zheng et al., 2013).

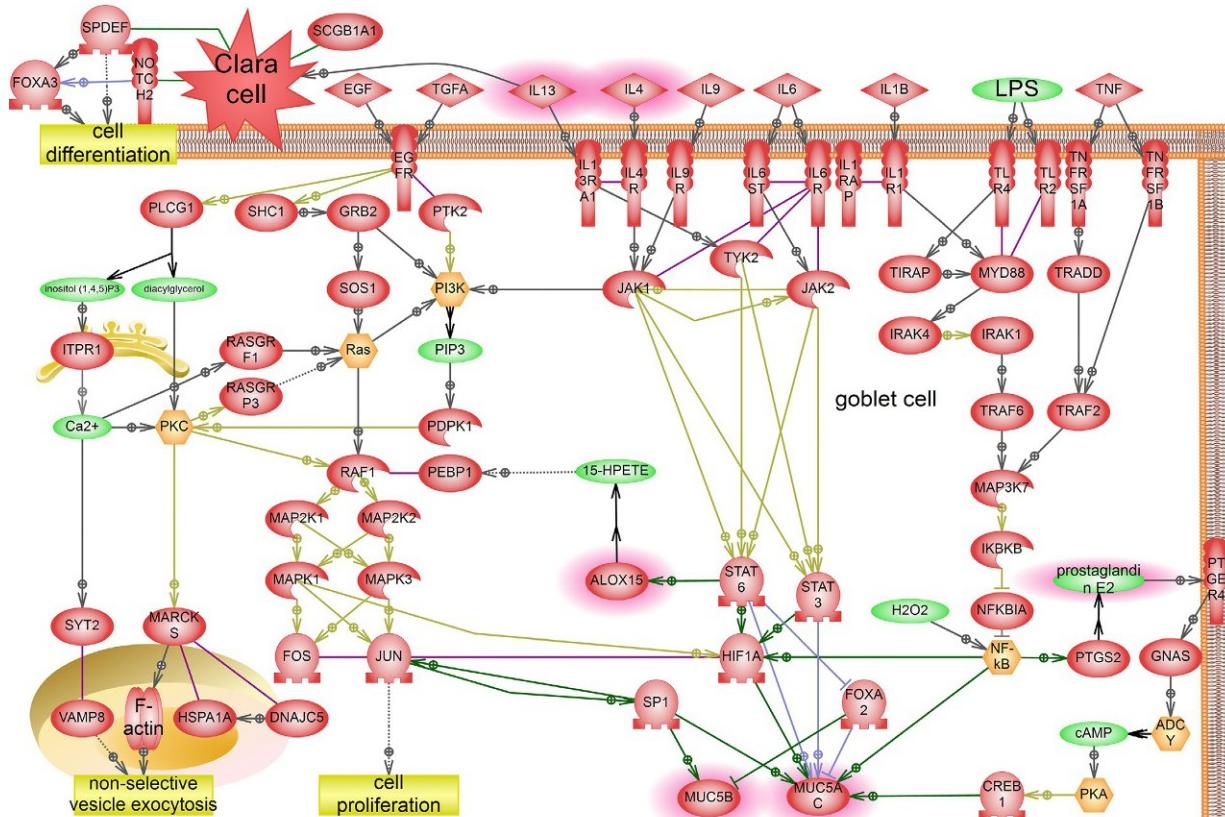
### **Regulation of airway surface liquid thickness (Fig. 6)**

Th2-type interleukins (IL-13, IL-6, IL-4, and IL-9) and Th17-type interleukins also induce the expression of a variety of ion channels and transporters (aquaporins (AQPs), chloride channel accessory (CLCA) proteins, the cystic fibrosis transmembrane regulator (CFTR)) and decrease the expression of the epithelial Na<sup>+</sup> channels (ENaC) in the respiratory epithelium (Aoki et al., 2014; Hollenhorst et al., 2011; Lennox et al., 2018).

Induced chloride (Cl<sup>-</sup>) secretion via CFTR and CLCA and decreased sodium (Na<sup>+</sup>) reabsorption via ENaC lead to the osmotic flow of water into the airway lumen and to an increase in airway surface liquid (ASL) thickness (Patel et al., 2009). A balanced ASL system is required for normal mucociliary clearance. Unlike asthma, hyperfunctioning of ENaC in cystic fibrosis leads to an increase in Na<sup>+</sup> absorption by the epithelium, thereby increasing osmotically driven water reabsorption and ASL dehydration (see cystic fibrosis). Both dehydration and fluid overflow cause airway dysfunction.

Furthermore, IL-13, IL-4, and IL-17 induce the expression of pendrin, also known as solute carrier family 26 member 4 (SLC26A4), in asthma and COPD mouse models, and it is likely that pendrin expression is enhanced in asthma patients as well (Izuhara et al., 2017; Nakao et al., 2008). SLC26A4 is a membrane protein that exchanges anions like bicarbonate,

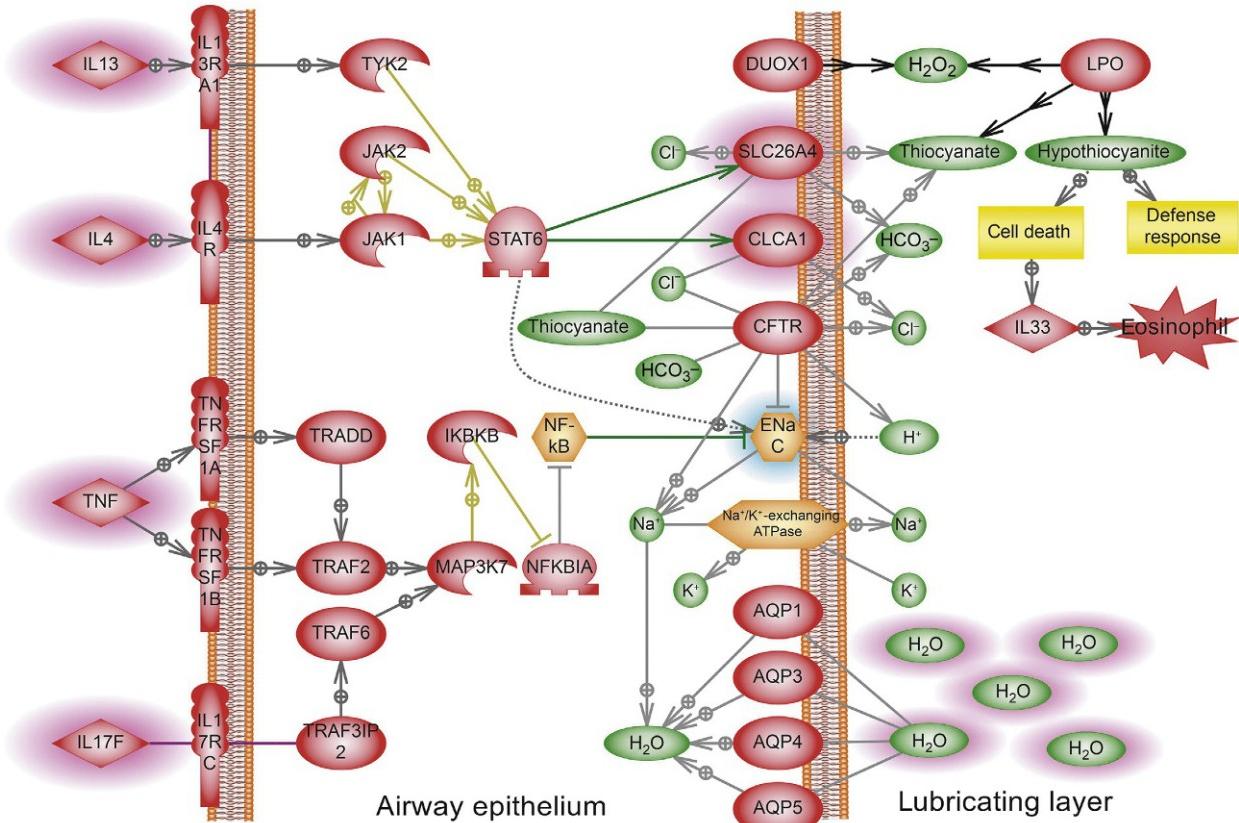
for chloride. Overexpressed SLC26A4 stimulates  $\text{Cl}^-/\text{HCO}_3^-$  exchange activity resulting in the reabsorption of water and decreased ASL thickness. SLC26A4 also somehow increases the production of mucin. In contrast with cystic fibrosis, where thiocyanite ( $\text{SCN}^-$ ) secretion is decreased, levels of SCN are increased in asthma perhaps due to SLC26A4 overexpression. Hypothiocyanite derived from thiocyanite is thought to have antimicrobial effects (see cystic fibrosis), but it may also be involved in the activation of NF-kB or even in cellular necrosis following release of IL-33, which accelerates eosinophilia in asthma ([Izuhara et al., 2017](#); [Nofziger et al., 2011](#)).



II. Human disease pathways

**FIG. 5** Pathway 3: Airway surface liquid (ASL) thickness and mucus accumulation in asthma. Production and release of mucus, mucus metaplasia.

## II. Human disease pathways



**FIG. 6** Pathway 3: Airway surface liquid (ASL) thickness and mucus accumulation in asthma. Regulation of airway surface liquid (ASL) thickness.

## Pathway 4

### Airway smooth muscle cells and airway remodeling in asthma

#### Incoming signals

In asthma, excessive shortening (contraction) of airway smooth muscle (ASM) cells and airway tissue remodeling together cause breathing difficulty.

Excessive narrowing of the airways and bronchoconstriction can be induced by the release of histamine, eicosanoids, and other mediators from mast cells, basophils, and eosinophils or by acetylcholine released from efferent parasympathetic nerves. Although the amount of released histamine or acetylcholine in allergic humans is not much different from healthy people, their ASMs have an increased sensitivity to bronchoconstrictors. The increased smooth muscle mass within the airway wall also stimulates bronchoconstriction even without a change in the intrinsic contractile properties of individual muscle cells ([Erle and Sheppard, 2014](#)).

IgE, Th2 cytokines, and other mediators from surrounding cells can modulate the hyperresponsiveness of asthmatic ASM cells.

#### Outcome

More data are needed to understand why airways overreact and narrowing occurs in response to triggers in patients with asthma. Existing hypotheses suggest several reasons for the observed overreaction including the increased mass or hypercontraction of asthmatic ASM cells and changes in the architecture of the epithelium and the ECM. Increased airway muscle mass and neovascularization result in remodeling of airway tissues. Hyperplasia or an increase in cellular proliferation, of ASM cells, is thought to be a more direct reason than hypertrophy for remodeling. Fibrosis, thickening of the airway basement membrane zone, increased deposition of ECM proteins, and other signs of tissue remodeling provoke further tissue injury ([Allen et al., 2012](#); [Ijpma et al., 2017](#); [West et al., 2013](#)).

#### Signaling

##### **Airway smooth muscle cell contraction ([Fig. 7](#))**

The main signaling pathway involved in ASM contraction is related to  $\text{Ca}^{2+}$ . The calcium pathways in ASM are induced by mediators acting mainly via G protein-coupled receptors (GPCRs). Phospholipase C beta1 (PLCB1) cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into inositol 1,2-diacylglycerol and trisphosphate (IP3), which in turn stimulate the release of  $\text{Ca}^{2+}$  from intracellular stores (such as those in the ER). In

addition to IP<sub>3</sub>, Ca<sup>2+</sup> release is stimulated by the activation of the CD38/RYR3 pathway or others yet to be discovered. Also, Ca<sup>2+</sup> can flow across the cellular plasma membrane through ion channels (not shown).

Ca<sup>2+</sup> transfers the activation signal to myosin light chain kinase (MYLK or MLCK) and RHOA. MYLK phosphorylates myosin light chain (MLC) leading to ASM contraction, which is terminated by MLC phosphatase (MLCP). RHOA activates Rho-associated coiled-coil-containing protein kinases 1 and 2 (ROCK 1 and ROCK 2) and the ROCKs directly phosphorylate and inactivate MLCP, allowing myosin phosphorylation to occur (Erle and Sheppard, 2014).

The reasons for ASM hypercontractility in patients with asthma remain unclear and are built on animal-based evidence. The proinflammatory cytokines IL-13, IL-1B, TNF, and IFNG, were shown to increase ASM contractility by facilitating CD38-mediated Ca<sup>2+</sup> release or RHOA expression (Berair et al., 2013).

Myosin light chain kinase (MYLK) is a candidate for the potentially affected or somehow abnormal protein, which causes ASM hypercontractility. MYLK expression levels were increased in patients with asthma and COPD compared with controls. The expression of ATPase sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> transporting 2 (ATP2A2), which reabsorbs calcium into the endoplasmic reticulum, is reduced in ASM cells in patients with asthma. The overexpression of ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3) is associated with the reduced ATP2A2 activity. Moreover, genetic variants in the region including ORMDL3 on chromosome 17q21 were related to childhood asthma risk. The expression of NADPH oxidase 4 (NOX4), an important source of ROS, which can regulate ATP2A2, was also increased in asthmatics ASM (Berair et al., 2013).

The regulation of ASM contraction and airway narrowing is depended on correct connections between cellular actin and myosin in the cytoskeleton of ASM with proteins in the extracellular matrix (ECM) (not shown in Fig. 7). Under conditions of chronic allergic reaction, the ECM architecture and structure changes toward fibrosis in asthma. Fibronectin, laminin, and collagen may trigger ASM cell proliferation in asthma by providing strengthened survival signals, probably via alpha-5beta-1 integrins (VLA-5) (see Fig. 8) (West et al., 2013; Wu et al., 2009).

### ***ASM proliferation and airway tissue remodeling (Fig. 8)***

It is presumed that ASM cell hyperplasia more than other cell types causes bronchial narrowing in asthma, although the published experimental data are not complete (Stewart, 2012).

In general, muscle cell proliferation is induced by mitogens acting mainly via tyrosine kinase receptors and G protein-coupled receptors (GPCRs). Stimulation of these receptors leads to the activation of the MAPKs and PI3K cascades. MAPK and PI3K-dependent signaling molecules

(AKT1, PAF1, RAC1, etc.) cooperate in the activation of transcription factors (SP1, JUN-FOS, etc.) required for cyclin D1 (CCND1) gene expression and in turn to cellular growth. Proliferation and cell cycle regulation are a complex process; only key points are shown in Fig. 8.

IgE was recently shown to induce ASM cell proliferation via the MAPK, AKT, and STAT3 signaling pathways. ASM cells express both FceRII/CD23 and FceRI receptors for IgE. Also, IgE signaling induces the release of Th2 and other mediators (including IL-4, IL-5, IL-13, IL-6, TNF, CCL11, and thymic stromal lymphopoietin (TSLP)) by ASM cells (not shown in Fig. 8) (Redhu et al., 2013a).

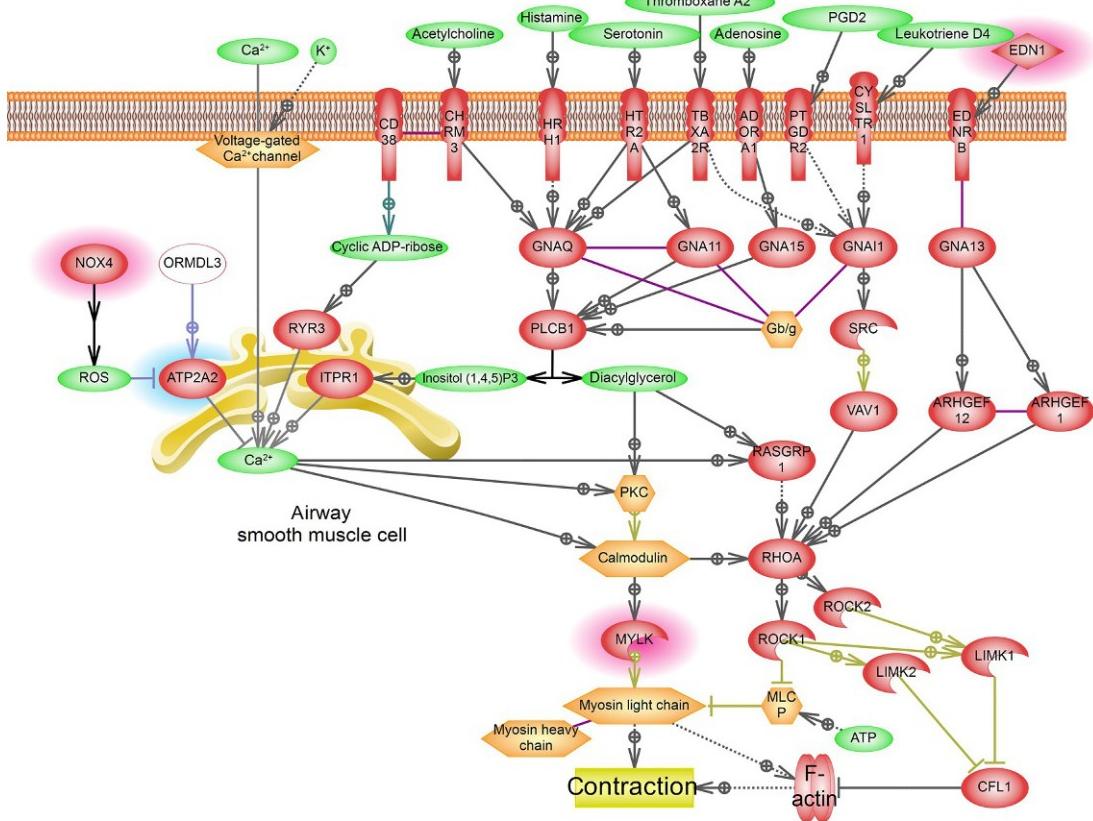
Inward migration and differentiation of external fibroblasts most likely contribute to the increase of ASM mass characteristic of asthma. Several signal proteins such as TGFB, platelet-derived growth factor subunit B (PDGFB), or CCL19 can induce fibroblast and myocyte migration and differentiation. Activated signaling associated with the pathology in bronchial epithelial cells plays an essential role in the maintenance of ASM proliferation in asthma. Mediators such as TSLP, IL-33, TGFB, endothelin 1 (EDN1), and cardiotrophin 1 (CTF1), released by irritated epithelium, also maintain the state of inflammation and tissue remodeling by supporting the production of Th2 cytokines released by immune cells (Deng et al., 2010; Erle and Sheppard, 2014).

For example, bronchial epithelial cells were shown to be a novel cell source (in addition to Th17 cells) of IL-17F in response to IL-33 signaling (Fujita et al., 2012). IL-13 promotes bronchial epithelial cells and lung fibroblasts to secrete high levels of periostin (POSTN) in asthma. Levels of POSTN expression are considered to be a biomarker for Th2 type, for eosinophilic asthma, and also for subepithelial fibrosis. POSTN enhances both fibrosis and profibrotic TGFB signaling in asthma (Jia et al., 2012; Parulekar et al., 2014).

TSLP is also highly expressed in ASM bundles from asthma and COPD patients. TSLP signaling enhances the release of proinflammatory mediators and chemoattractants for leukocytes by epithelial and ASM cells. Moreover, TSLP induces migration and cytoskeletal reorganization in ASM cells and in dendritic cells. The inflammatory mediators IL-1B and TNFA regulate human TSLP gene expression (Redhu et al., 2013b).

Lastly, the expression of the ADAM metallopeptidase domain 33 (ADAM33) protein is increased in smooth muscle, epithelial, and mesenchymal cells of asthmatic patients. Polymorphisms in the ADAM33 gene are also significantly associated with asthma. ADAM33 acts as a cell surface membrane-bound enzyme (termed sheddase) that cleaves extracellular portions of transmembrane receptors, thereby modifying their signaling characteristics. ADAM33 may be involved in airway remodeling through the regulation of cellular differentiation and neoangiogenesis. TGFB1 can influence the function of ADAM33 (Sharma et al., 2011; Vishweswaraiah et al., 2014).

## II. Human disease pathways



**FIG. 7** Pathway 4: Airway smooth muscle cells and airway remodeling in asthma. Airway smooth muscle cells contraction.

## II. Human disease pathways

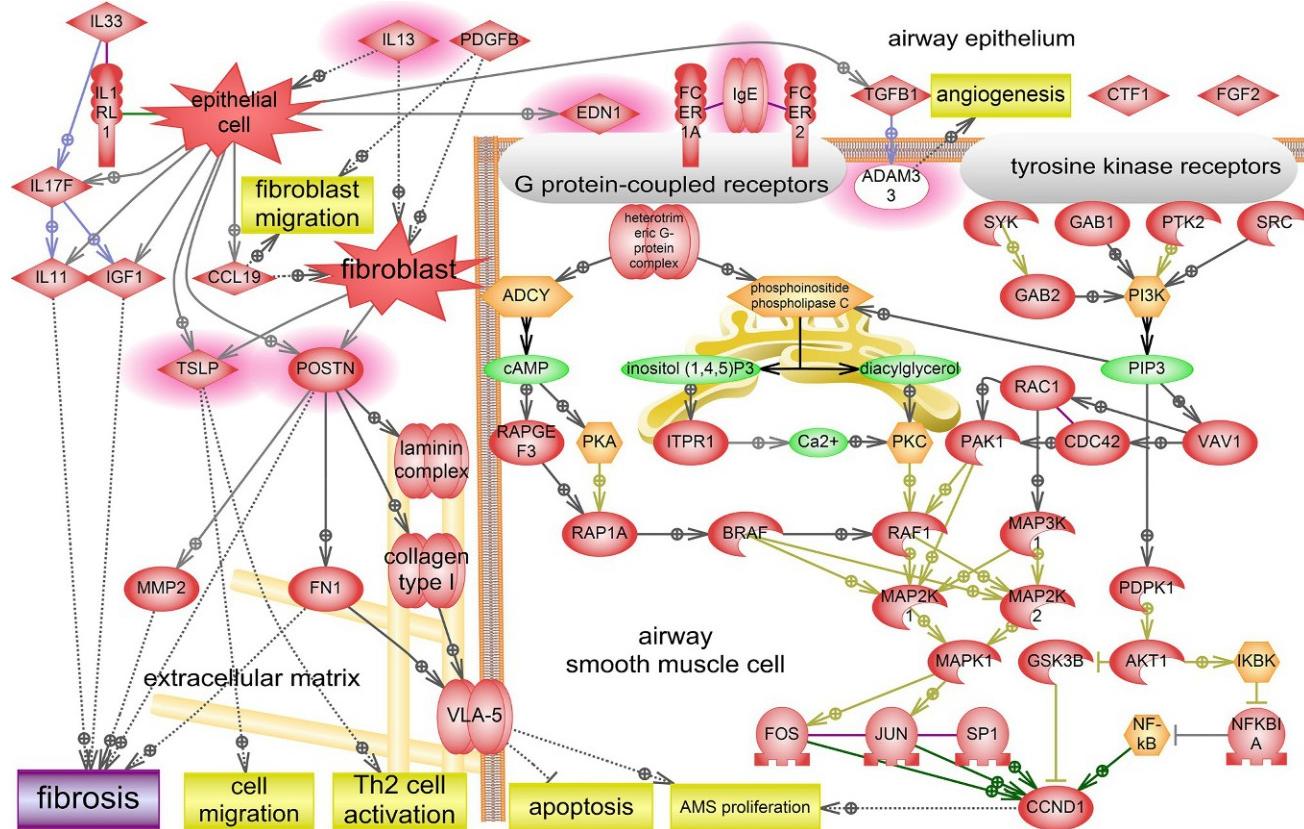


FIG. 8 Pathway 4: Airway smooth muscle cells and airway remodeling in asthma. ASM proliferation and airway tissue remodeling.

## References

- Disease numbers # 600807 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code J45. Diseases of the respiratory system (J00-J99). (ICD-10, <https://icdlist.com>). ICD-11: disease code CA23.
- Adachi, T., Alam, R., 1998. The mechanism of IL-5 signal transduction. *Am. J. Phys.* 275, C623-C633.
- Adachi, T., Vita, R., Sannohe, S., Stafford, S., Alam, R., Kayaba, H., Chihara, J., 2001. The functional role of rho and rho-associated coiled-coil forming protein kinase in eotaxin signaling of eosinophils. *J. Immunol.* 167, 4609–4615. <https://doi.org/10.4049/jimmunol.167.8.4609>.
- Akaba, T., Komiya, K., Suzuki, I., Kozaki, Y., Tamaoki, J., Rubin, B.K., 2018. Activating prostaglandin E2 receptor subtype EP4 increases secreted mucin from airway goblet cells. *Pulm. Pharmacol. Ther.* 48, 117–123. <https://doi.org/10.1016/j.pupt.2017.11.001>.
- Al-Ghouleh, A., Johal, R., Sharquie, I.K., Emara, M., Harrington, H., Shakib, F., Ghaemmaghami, A.M., 2012. The glycosylation pattern of common allergens: the recognition and uptake of Der p 1 by epithelial and dendritic cells is carbohydrate dependent. *PLoS One* 7, e33929. <https://doi.org/10.1371/journal.pone.0033929>.
- Allen, J.C., Seidel, P., Schlosser, T., Ramsay, E.E., Ge, Q., Ammit, A.J., 2012. Cyclin D1 in ASM cells from asthmatics is insensitive to corticosteroid inhibition. *J. Allergy* 2012, 1–6. <https://doi.org/10.1155/2012/307838>.
- Aoki, H., Mogi, C., Okajima, F., 2014. Ionotropic and metabotropic proton-sensing receptors involved in airway inflammation in allergic asthma. *Mediat. Inflamm.* 2014, 1–8. <https://doi.org/10.1155/2014/712962>.
- Barthel, S.R., Johansson, M.W., McNamee, D.M., Mosher, D.F., 2008. Roles of integrin activation in eosinophil function and the eosinophilic inflammation of asthma. *J. Leukoc. Biol.* 83, 1–12. <https://doi.org/10.1189/jlb.0607344>.
- Beekman, J.M., Verhagen, L.P., Geijzen, N., Coffer, P.J., 2009. Regulation of myelopoiesis through syntenin-mediated modulation of IL-5 receptor output. *Blood* 114, 3917–3927. <https://doi.org/10.1182/blood-2009-03-208850>.
- Berair, R., Hollins, F., Brightling, C., 2013. Airway smooth muscle hypercontractility in asthma. *J. Allergy* 2013, 1–7. <https://doi.org/10.1155/2013/185971>.
- Besnard, A.-G., Togbe, D., Guillou, N., Erard, F., Quesniaux, V., Ryffel, B., 2011. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur. J. Immunol.* 41, 1675–1686. <https://doi.org/10.1002/eji.201041033>.
- Bonser, L.R., Erle, D.J., 2017. Airway mucus and asthma: the role of MUC5AC and MUC5B. *J. Clin. Med.* 6, <https://doi.org/10.3390/jcm6120112>.
- Burgel, P.-R., Nadel, J.A., 2008. Epidermal growth factor receptor-mediated innate immune responses and their roles in airway diseases. *Eur. Respir. J.* 32, 1068–1081. <https://doi.org/10.1183/09031936.00172007>.
- Chan, M.A., Gigliotti, N.M., Aubin, B.G., Rosenwasser, L.J., 2014. FCER2 (CD23) asthma-related single nucleotide polymorphisms yields increased IgE binding and Egr-1 expression in human B cells. *Am. J. Respir. Cell Mol. Biol.* 50, 263–269. <https://doi.org/10.1165/rcmb.2013-0112OC>.
- Chen, G., Korfhagen, T.R., Xu, Y., Kitzmiller, J., Wert, S.E., Maeda, Y., Gregorieff, A., Clevers, H., Whitsett, J.A., 2009. SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. *J. Clin. Invest.* <https://doi.org/10.1172/JCI39731>.
- Coleman, J.M., Naik, C., Holguin, F., Ray, A., Ray, P., Trudeau, J.B., Wenzel, S.E., 2012. Epithelial eotaxin-2 and eotaxin-3 expression: relation to asthma severity, luminal eosinophilia and age at onset. *Thorax* 67, 1061–1066. <https://doi.org/10.1136/thoraxjnl-2012-201634>.

- Davis, B.K., Wen, H., Ting, J.P.-Y., 2011. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu. Rev. Immunol.* 29, 707–735. <https://doi.org/10.1146/annurev-immunol-031210-101405>.
- Deng, H., Hershenson, M.B., Lei, J., Bitar, K.N., Fingar, D.C., Solway, J., Bentley, J.K., 2010. p70 ribosomal S6 kinase is required for airway smooth muscle cell size enlargement but not increased contractile protein expression. *Am. J. Respir. Cell Mol. Biol.* 42, 744–752. <https://doi.org/10.1165/rcmb.2009-0037OC>.
- Erle, D.J., Sheppard, D., 2014. The cell biology of asthma. *J. Cell Biol.* 205, 621–631. <https://doi.org/10.1083/jcb.201401050>.
- Fang, S., Crews, A.L., Chen, W., Park, J., Yin, Q., Ren, X.-R., Adler, K.B., 2013. MARCKS and HSP70 interactions regulate mucin secretion by human airway epithelial cells in vitro. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 304, L511–L518. <https://doi.org/10.1152/ajplung.00337.2012>.
- Feng, Y.-H., Mao, H., 2012. Specific regulator of eosinophil apoptosis: Siglec-8-new hope for bronchial asthma treatment. *Clin. Med. J.* 125, 2048–2052.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Fujita, J., Kawaguchi, M., Kokubu, F., Ohara, G., Ota, K., Huang, S.-K., Morishima, Y., Ishii, Y., Satoh, H., Sakamoto, T., Hizawa, N., 2012. Interleukin-33 induces interleukin-17F in bronchial epithelial cells. *Allergy* 67, 744–750. <https://doi.org/10.1111/j.1365-9995.2012.02825.x>.
- Fyhrquist-Vanni, N., Alenius, H., Lauerma, A., 2007. Contact dermatitis. *Dermatol. Clin., Cutaneous Receptors: Clinical Implications and Therapeutic Relevance* 25, 613–623. <https://doi.org/10.1016/j.det.2007.06.002>.
- García, L.N., Leimgruber, C., Uribe Echevarría, E.M., Acosta, P.L., Brahamian, J.M., Polack, F.P., Miró, M.S., Quintar, A.A., Sotomayor, C.E., Maldonado, C.A., 2014. Protective phenotypes of club cells and alveolar macrophages are favored as part of endotoxin-mediated prevention of asthma. *Exp. Biol. Med.* <https://doi.org/10.1177/1535370214562338>.
- Hadebe, S., Brombacher, F., Brown, G.D., 2018. C-type lectin receptors in asthma. *Front. Immunol.* 9, 733. <https://doi.org/10.3389/fimmu.2018.00733>.
- Hahn, C., Islamian, A.P., Renz, H., Nockher, W.A., 2006. Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation. *J. Allergy Clin. Immunol.* 117, 787–794. <https://doi.org/10.1016/j.jaci.2005.12.1339>.
- Hall, S., Agrawal, D.K., 2014. Key mediators in the immunopathogenesis of allergic asthma. *Int. Immunopharmacol.* 23, 316–329. <https://doi.org/10.1016/j.intimp.2014.05.034>.
- Hall, S.C., Agrawal, D.K., 2016. Toll-like receptors, triggering receptor expressed on myeloid cells family members and receptor for advanced glycation end-products in allergic airway inflammation. *Expert Rev. Respir. Med.* 10, 171–184. <https://doi.org/10.1586/17476348.2016.1133303>.
- Hollenhorst, M.I., Richter, K., Fronius, M., 2011. Ion transport by pulmonary epithelia. *J. Biomed. Biotechnol.* 2011, 1–16. <https://doi.org/10.1155/2011/174306>.
- Houimel, M., Mazzucchelli, L., 2013. Chemokine CCR3 ligands-binding peptides derived from a random phage-epitope library. *Immunol. Lett.* 149, 19–29. <https://doi.org/10.1016/j.imlet.2012.11.006>.
- Ijpma, G., Panariti, A., Lauzon, A.-M., Martin, J.G., 2017. Directional preference of airway smooth muscle mass increase in human asthmatic airways. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 312, L845–L854. <https://doi.org/10.1152/ajplung.00353.2016>.
- Isgrò, M., Bianchetti, L., Marini, M.A., Bellini, A., Schmidt, M., Mattoli, S., 2013. The C-C motif chemokine ligands CCL5, CCL11, and CCL24 induce the migration of circulating fibrocytes from patients with severe asthma. *Mucosal Immunol.* 6, 718–727. <https://doi.org/10.1038/mi.2012.109>.
- Izuhara, K., Suzuki, S., Ogawa, M., Nunomura, S., Nanri, Y., Mitamura, Y., Yoshihara, T., 2017. The significance of hypothiocyanite production via the pendrin/DUOX/peroxidase pathway in the pathogenesis of asthma. *Oxidative Med. Cell. Longev.* 2017, 1054801. <https://doi.org/10.1155/2017/1054801>.

- Jia, G., Erickson, R.W., Choy, D.F., Mosesova, S., Wu, L.C., Solberg, O.D., Shikotra, A., Carter, R., Audusseau, S., Hamid, Q., Bradding, P., Fahy, J.V., Woodruff, P.G., Harris, J.M., Arron, J.R., Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (BOBCAT) Study Group, 2012. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J. Allergy Clin. Immunol.* 130, 647–654. e10. <https://doi.org/10.1016/j.jaci.2012.06.025>.
- Jones, L.C., Moussa, L., Fulcher, M.L., Zhu, Y., Hudson, E.J., O'Neal, W.K., Randell, S.H., Lazarowski, E.R., Boucher, R.C., Kreda, S.M., 2012. VAMP8 is a vesicle SNARE that regulates mucin secretion in airway goblet cells. *J. Physiol.* 590, 545–562. <https://doi.org/10.1113/jphysiol.2011.222091>.
- Joseph, J., Benedict, S., Safa, W., Joseph, M., 2004. Serum interleukin-5 levels are elevated in mild and moderate persistent asthma irrespective of regular inhaled glucocorticoid therapy. *BMC Pulm. Med.* 4, 2. <https://doi.org/10.1186/1471-2466-4-2>.
- Kaiko, G.E., Horvat, J.C., Beagley, K.W., Hansbro, P.M., 2008. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* 123, 326–338. <https://doi.org/10.1111/j.1365-2567.2007.02719.x>.
- Lai, H.Y., Rogers, D.F., 2010. Mucus hypersecretion in asthma: intracellular signalling pathways as targets for pharmacotherapy. *Curr. Opin. Allergy Clin. Immunol.* 10, 67–76. <https://doi.org/10.1097/ACI.0b013e328334643a>.
- Lennox, A.T., Coburn, S.L., Leech, J.A., Heidrich, E.M., Kleyman, T.R., Wenzel, S.E., Pilewski, J.M., Corcoran, T.E., Myerburg, M.M., 2018. ATP12A promotes mucus dysfunction during Type 2 airway inflammation. *Sci. Rep.* 8, 2109. <https://doi.org/10.1038/s41598-018-20444-8>.
- Lindén, A., Dahlén, B., 2014. Interleukin-17 cytokine signalling in patients with asthma. *Eur. Respir. J.* 44, 1319–1331. <https://doi.org/10.1183/09031936.00002314>.
- Loke, I., Kolarich, D., Packer, N.H., Thaysen-Andersen, M., 2016. Emerging roles of protein mannosylation in inflammation and infection. *Mol. Asp. Med.* 51, 31–55. <https://doi.org/10.1016/j.mam.2016.04.004>.
- Matsushita, K., Yoshimoto, T., 2014. B cell-intrinsic MyD88 signaling is essential for IgE responses in lungs exposed to pollen allergens. *J. Immunol.* 193, 5791–5800. <https://doi.org/10.4049/jimmunol.1401768>.
- Milovanovic, M., Volarevic, V., Radosavljevic, G., Jovanovic, I., Pejnovic, N., Arsenijevic, N., Lukic, M.L., 2012. IL-33/ST2 axis in inflammation and immunopathology. *Immunol. Res.* 52, 89–99. <https://doi.org/10.1007/s12026-012-8283-9>.
- Murphy, J.M., Young, I.G., 2006. IL-3, IL-5, and GM-CSF signaling: crystal structure of the human beta-common receptor. In: Vitamins & Hormones, Interleukins. Academic Press, pp. 1–30. [https://doi.org/10.1016/S0083-6729\(06\)74001-8](https://doi.org/10.1016/S0083-6729(06)74001-8).
- Nakao, I., Kanaji, S., Ohta, S., Matsushita, H., Arima, K., Yuyama, N., Yamaya, M., Nakayama, K., Kubo, H., Watanabe, M., Sagara, H., Sugiyama, K., Tanaka, H., Toda, S., Hayashi, H., Inoue, H., Hoshino, T., Shiraki, A., Inoue, M., Suzuki, K., Aizawa, H., Okinami, S., Nagai, H., Hasegawa, M., Fukuda, T., Green, E.D., Izuhara, K., 2008. Identification of pendrin as a common mediator for mucus production in bronchial asthma and chronic obstructive pulmonary disease. *J. Immunol.* 180, 6262–6269.
- Nechama, M., Kwon, J., Wei, S., Kyi, A.T., Welner, R.S., Ben-Dov, I.Z., Arredouani, M.S., Asara, J.M., Chen, C.-H., Tsai, C.-Y., Nelson, K.F., Kobayashi, K.S., Israel, E., Zhou, X.Z., Nicholson, L.K., Lu, K.P., 2018. The IL-33-PIN1-IRAK-M axis is critical for type 2 immunity in IL-33-induced allergic airway inflammation. *Nat. Commun.* 9, <https://doi.org/10.1038/s41467-018-03886-6>.
- Nofziger, C., Dossena, S., Suzuki, S., Izuhara, K., Paulmichl, M., 2011. Pendrin function in airway epithelia. *Cell. Physiol. Biochem.* 28, 571–578. <https://doi.org/10.1159/000335115>.
- Ota, K., Kawaguchi, M., Matsukura, S., Kurokawa, M., Kokubu, F., Fujita, J., Morishima, Y., Huang, S.-K., Ishii, Y., Satoh, H., Hizawa, N., 2014. Potential involvement of IL-17F in asthma. *J Immunol Res* 2014, 1–8. <https://doi.org/10.1155/2014/602846>.

- Parulekar, A.D., Atik, M.A., Hanania, N.A., 2014. Periostin, a novel biomarker of TH2-driven asthma. *Curr. Opin. Pulm. Med.* 20, 60–65. <https://doi.org/10.1097/MCP.0000000000000005>.
- Patel, A.C., Brett, T.J., Holtzman, M.J., 2009. The role of CLCA proteins in inflammatory airway disease. *Annu. Rev. Physiol.* 71, 425–449. <https://doi.org/10.1146/annurev.physiol.010908.163253>.
- Pelaia, G., Vatrella, A., Busceti, M.T., Gallelli, L., Calabrese, C., Terracciano, R., Maselli, R., 2015. Cellular mechanisms underlying eosinophilic and neutrophilic airway inflammation in asthma. *Mediat. Inflamm.* 2015, 879783. <https://doi.org/10.1155/2015/879783>.
- Perrais, M., Pigny, P., Copin, M.-C., Aubert, J.-P., Van Seuningen, I., 2002. Induction of MUC2 and MUC5AC mucins by factors of the epidermal growth factor (EGF) family is mediated by EGF receptor/Ras/Raf/extracellular signal-regulated kinase cascade and Sp1. *J. Biol. Chem.* 277, 32258–32267. <https://doi.org/10.1074/jbc.M204862200>.
- Provost, V., Larose, M.-C., Langlois, A., Rola-Pleszczynski, M., Flamand, N., Laviolette, M., 2013. CCL26/eotaxin-3 is more effective to induce the migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2. *J. Leukoc. Biol.* 94, 213–222. <https://doi.org/10.1189/jlb.0212074>.
- Rahman, M.M., Alkhouri, H., Tang, F., Che, W., Ge, Q., Ammit, A.J., 2014. Sphingosine 1-phosphate induces neutrophil chemoattractant IL-8: repression by steroids. *PLoS One* 9, e92466. <https://doi.org/10.1371/journal.pone.0092466>.
- Raiford, K.L., Park, J., Lin, K.-W., Fang, S., Crews, A.L., Adler, K.B., 2011. Mucin granule-associated proteins in human bronchial epithelial cells: the airway goblet cell “granulome”. *Respir. Res.* 12, 118. <https://doi.org/10.1186/1465-9921-12-118>.
- Rajaram, M.V.S., Arnett, E., Azad, A.K., Guirado, E., Ni, B., Gerberick, A.D., He, L.-Z., Keler, T., Thomas, L.J., Lafuse, W.P., Schlesinger, L.S., 2017. *M. tuberculosis*-initiated human mannose receptor signaling regulates macrophage recognition and vesicle trafficking by FcR $\gamma$ -chain, Grb2, and SHP-1. *Cell Rep.* 21, 126–140. <https://doi.org/10.1016/j.celrep.2017.09.034>.
- Reader, J.R., Tepper, J.S., Schelegle, E.S., Aldrich, M.C., Putney, L.F., Pfeiffer, J.W., Hyde, D.M., 2003. Pathogenesis of mucous cell metaplasia in a murine asthma model. *Am. J. Pathol.* 162, 2069–2078. [https://doi.org/10.1016/S0002-9440\(10\)64338-6](https://doi.org/10.1016/S0002-9440(10)64338-6).
- Redhu, N.S., Shan, L., Al-Subait, D., Ashdown, H.L., Movassagh, H., Lamkhioued, B., Gounni, A.S., 2013a. IgE induces proliferation in human airway smooth muscle cells: role of MAPK and STAT3 pathways. *Allergy Asthma Clin. Immunol. Off. J. Can. Soc. Allergy Clin. Immunol.* 9, 41. <https://doi.org/10.1186/1710-1492-9-41>.
- Redhu, N.S., Shan, L., Movassagh, H., Gounni, A.S., 2013b. Thymic stromal lymphopoietin induces migration in human airway smooth muscle cells. *Sci. Rep.* 3, <https://doi.org/10.1038/srep02301>.
- Rogers, D.F., 2004. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr. Opin. Pharmacol.* 4, 241–250. <https://doi.org/10.1016/j.coph.2004.01.011>.
- Sharma, N., Tripathi, P., Awasthi, S., 2011. Role of ADAM33 gene and associated single nucleotide polymorphisms in asthma. *Allergy Rhinol.* 2, e63–e70. <https://doi.org/10.2500/ar.2011.2.0018>.
- Shen, Z.-J., Malter, J.S., 2015. Determinants of eosinophil survival and apoptotic cell death. *Apoptosis Int. J. Program. Cell Death* 20, 224–234. <https://doi.org/10.1007/s10495-014-1072-2>.
- Stewart, A., 2012. More muscle in asthma, but where did it come from? *Am. J. Respir. Crit. Care Med.* 185, 1035–1037. <https://doi.org/10.1164/rccm.201203-0457ED>.
- Stolarski, B., Kurowska-Stolarska, M., Kewin, P., Xu, D., Liew, F.Y., 2010. IL-33 exacerbates eosinophil-mediated airway inflammation. *J. Immunol.* 185, 3472–3480. <https://doi.org/10.4049/jimmunol.1000730>.
- Tang, W., Smith, S.G., Du, W., Gugilla, A., Du, J., Oliveria, J.P., Howie, K., Salter, B.M., Gauvreau, G.M., O'Byrne, P.M., Sehmi, R., 2018. Interleukin-25 and eosinophils progenitor cell mobilization in allergic asthma. *Clin. Transl. Allergy* 8, 5. <https://doi.org/10.1186/s13601-018-0190-2>.

- Vishweswaraiah, S., Veerappa, A.M., Mahesh, P.A., Jayaraju, B.S., Krishnarao, C.S., Ramachandra, N.B., 2014. Molecular interaction network and pathway studies of ADAM33 potentially relevant to asthma. *Ann. Allergy Asthma Immunol. Off. Publ. Am. Coll. Allergy Asthma Immunol.* 113, 418–424. e1. <https://doi.org/10.1016/j.anai.2014.07.009>.
- Volckaert, T., De Langhe, S., 2014. Lung epithelial stem cells and their niches: Fgf10 takes center stage. *Fibrogenesis Tissue Repair* 7, 8. <https://doi.org/10.1186/1755-1536-7-8>.
- West, E.E., Kashyap, M., Leonard, W.J., 2012. TSLP: a key regulator of asthma pathogenesis. *Drug Discov. Today Dis. Mech.* 9, e83–e88. <https://doi.org/10.1016/j.ddmec.2012.09.003>.
- West, A.R., Syyong, H.T., Siddiqui, S., Pascoe, C.D., Murphy, T.M., Maarsingh, H., Deng, L., Maksym, G.N., Bossé, Y., 2013. Airway contractility and remodeling: links to asthma symptoms. *Pulm. Pharmacol. Ther.* 26, 3–12. <https://doi.org/10.1016/j.pupt.2012.08.009>.
- Williams, O.W., Sharafkhaneh, A., Kim, V., Dickey, B.F., Evans, C.M., 2006. Airway mucus: from production to secretion. *Am. J. Respir. Cell Mol. Biol.* 34, 527–536. <https://doi.org/10.1165/rcmb.2005-0436SF>.
- Wong, C.K., Cheung, P.F.Y., Ip, W.K., Lam, C.W.K., 2005. Interleukin-25-induced chemokines and interleukin-6 release from eosinophils is mediated by p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and nuclear factor-kappaB. *Am. J. Respir. Cell Mol. Biol.* 33, 186–194. <https://doi.org/10.1165/rcmb.2005-0034OC>.
- Wu, H., Romieu, I., Sienra-Monge, J.-J., Li, H., del Rio-Navarro, B.E., London, S.J., 2009. Genetic variation in ORML1-like 3 (ORMDL3) and gasdermin-like (GSDML) and childhood asthma. *Allergy* 64, 629–635. <https://doi.org/10.1111/j.1398-9995.2008.01912.x>.
- Wu, S., Li, H., Yu, L., Wang, N., Li, X., Chen, W., 2017. IL-1 $\beta$  upregulates Muc5ac expression via NF- $\kappa$ B-induced HIF-1 $\alpha$  in asthma. *Immunol. Lett.* 192, 20–26. <https://doi.org/10.1016/j.imlet.2017.10.006>.
- Yang, Z., Robinson, M.J., Allen, C.D.C., 2014. Regulatory constraints in the generation and differentiation of IgE-expressing B cells. *Curr. Opin. Immunol.* 28, 64–70. <https://doi.org/10.1016/j.coic.2014.02.001>.
- Zhao, J., Maskrey, B., Balzar, S., Chibana, K., Mustovich, A., Hu, H., Trudeau, J.B., O'Donnell, V., Wenzel, S.E., 2009. Interleukin-13-induced MUC5AC is regulated by 15-lipoxygenase 1 pathway in human bronchial epithelial cells. *Am. J. Respir. Crit. Care Med.* 179, 782–790. <https://doi.org/10.1164/rccm.200811-1744OC>.
- Zhao, J., O'Donnell, V.B., Balzar, S., St Croix, C.M., Trudeau, J.B., Wenzel, S.E., 2011. 15-Lipoxygenase 1 interacts with phosphatidylethanolamine-binding protein to regulate MAPK signaling in human airway epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 108, 14246–14251. <https://doi.org/10.1073/pnas.1018075108>.
- Zheng, D., Limmon, G.V., Yin, L., Leung, N.H.N., Yu, H., Chow, V.T.K., Chen, J., 2013. A cellular pathway involved in Clara cell to alveolar type II cell differentiation after severe lung injury. *PLoS One* 8, e71028. <https://doi.org/10.1371/journal.pone.0071028>.

## CHAPTER

## 9.2

## Chronic obstructive pulmonary disease

The pathophysiology of chronic obstructive pulmonary disease is related to an enhanced inflammatory response to noxious particles and gases, chronic airway irritation, mucus production, and pulmonary scarring and changes in the pulmonary vasculature.

Chronic obstructive pulmonary disease (COPD) is an inflammatory respiratory disease usually caused by exposure to tobacco smoke. (*Ferri and Ferri, 2018*).

Traditionally, COPD was described as encompassing both pulmonary emphysema, characterized by the loss of lung elasticity, and the destruction of lung parenchyma with enlargement of air spaces and chronic bronchitis, characterized by obstruction of small airways and the persistence of a productive cough for more than 3 months over the course of 2 successive years. These terms are no longer included in the formal definition of COPD, although they are still used clinically. Although emphysema and chronic bronchitis are commonly associated with COPD, neither is required to support a diagnosis of COPD (*Ferri and Ferri, 2018*).

Alpha-1 antitrypsin deficiency (see *Chapter 2.3 Endocrine, Nutritional, and Metabolic diseases*), which often remains undiagnosed, can be a cause of COPD (Genetics Home Reference, <https://ghr.nlm.nih.gov>). There are several key cells affected in COPD, including alveolar epithelial cells, alveolar macrophages, T cells, neutrophils, goblet and mucous cells, fibroblasts, and some other cells. Cigarette smoke is considered as one of a primary causative factor in COPD development.

Alveolar epithelial cells exposed to cigarette smoke often undergo apoptosis, develop impaired DNA repair, undergo autophagy or express the ER-stress response, and produce inflammatory mediators and proteases. Altogether, cigarette smoke provokes destruction of the alveolar septum and promotes the development of pulmonary emphysema: **Pathway 1.** *Cigarette smoke causes dysfunction and production of inflammatory mediators in alveolar epithelial cells (Fig. 9).*

**Pathway 2.** *Cigarette smoke and inflammation cause alveolar epithelial cell death in COPD (Fig. 10).*

The suppression of alveolar macrophages is decreased in COPD, so pathologically high amounts of active macrophages drive the inflammatory progression and consequent pulmonary tissue destruction:

**Pathway 3.** *Role of alveolar macrophages in pulmonary tissue destruction in COPD* ([Fig. 11](#)).

Goblet and mucous cells are hyperstimulated in COPD and produce excess amounts of mucus, leading to an increased predisposition to infection and inflammation:

**Pathway 4.** *Mucus hyperproduction in goblet and mucous cells in COPD* ([Fig. 12](#)).

## Key cellular contributors and processes

Alveolar epithelial cell

Cell

The alveolar epithelial cells (pneumocytes) line the alveolar compartment of the lungs. There exist two types of alveolar cells: type I (the prevailing type) and type II alveolar cells. Type I alveolar cells are squamous extremely thin cells involved in the process of gas exchange between the alveoli and blood. Type II alveolar cells are involved in the secretion of surfactant proteins.

Alveolar macrophages

Cell

The alveolar macrophages are a type of macrophages found in the pulmonary alveolus, near the alveolar epithelial cells. The alveolar macrophages are cells of the innate immune system; they remove various infectious or allergic particles from the respiratory surfaces and represent the first line of defense against airborne pathogens.

Apoptosis

Process

Apoptosis is a highly regulated chain of events leading to cell destruction that occurs in multicellular organisms. Apoptosis eliminates damaged or redundant cells and is required for normal tissue development and homeostasis.

Autophagy

Process

Autophagy is a conserved eukaryotic process in which excessive or dysfunctional intracellular components are delivered to lysosomes for degradation. The three major types of autophagy include macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membrane vesicle, the autophagosome.

Chemokines

Protein or gene

Chemokines are a family of a larger group of extracellular signaling molecules called cytokines. Chemokines are secreted low-molecular-weight proteins, which can induce chemotaxis—directed movement of a cell in response to a molecular stimulus.

## Cytokines

### Protein or gene

Cytokines are a broad list of small proteins released by immune cells, which participate in cell-to-cell communication and regulate immune responses. Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors.

## Endoplasmic reticulum stress response

### Process

The endoplasmic reticulum protein response (unfolded protein response, UPR) is a highly conserved adaptive process in eukaryotes triggered by a buildup of unfolded and/or misfolded proteins in the endoplasmic reticulum lumen. The UPR leads to restoration of normal cellular functioning or elimination of a severely damaged cell via apoptosis.

## Goblet cells

### Cell

Goblet cells are columnar epithelial cells that secrete gel-forming proteins called mucins (major constituents of mucus). Goblet cells are typically found in the epithelial lining of organs, for example, the respiratory and gastrointestinal tracts, and are surrounded by stratified squamous cells.

## Matrix metalloproteinases

### Protein or gene

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases. These enzymes are responsible for processing and degrading most of the constituents of the extracellular matrix. Their targets include a variety of extracellular proteins and other molecules, and thus MMPs can be viewed as regulators of key cellular and tissue processes.

## Pathogen pattern recognition

### Protein or gene

During the initial stage of response to infection, the innate defense system employs pattern recognition receptors (PRRs) that recognize components of invading pathogens. The PRRs are able to detect evolutionarily conserved molecular structures called pathogen-associated molecular patterns (PAMPs) derived from bacteria and viruses and initiate an antimicrobial inflammatory response. At the same time, PRRs can also detect damage-associated molecular patterns (DAMPs) released from host cells during cell damage or death and initiate a noninfectious inflammatory response.

## Phagocytosis

### Process

Phagocytosis is a form of endocytosis by which a cell internalizes large ( $>0.5\text{ }\mu\text{m}$ ) particles via the formation of an internal compartment known as a phagosome, which is further fused with a lysosome for degradation. Professional immune cells (macrophages, neutrophils, and others) employ phagocytosis to remove invading pathogens.

## Pathway 1

### Cigarette smoke causes dysfunction and production of inflammatory mediators in alveolar epithelial cells (Fig. 9)

#### Incoming signals

The effect of cigarette smoke (i.e., tobacco exposure) on alveolar type I epithelial cells is one of the leading factors in COPD development. Cigarette smoke modulates the toll-like receptor 4 (TLR4), advanced glycosylation end product-specific receptor (AGER), MAPK1/3, and sirtuin 1 (SIRT1) cascades leading to either the synthesis of proinflammatory cytokines or alveolar epithelial cell death. Damage to alveolar epithelial cells leads to the liberation of different intracellular molecules and fragments of molecules into the extracellular space that in turn triggers subsequent damage to the following cell types.

#### Outcome effects

Cigarette smoke can activate epithelial cells to secrete a variety of inflammatory mediators and proteases. Inflammatory mediators are responsible for macrophage, neutrophil, and T-cell recruitment. Transforming growth factor beta 1 (TGFB1) plays a significant role in the induction of pulmonary fibrosis. Proteases are responsible for extracellular matrix degradation that contributes to alveolar septal destruction. Low levels of NAD-dependent deacetylases (sirtuins) activity contribute to alveolar epithelial cell dysfunction.

#### Signaling

Cigarette smoke was shown to induce TLR4 and AGER signaling, which causes high levels of expression of many proinflammatory proteins. AGER may also elevate the expression of itself in the airway epithelium of COPD patients.

Specific endogenous molecules released by dying cells (damage-associated molecular patterns, DAMPs) bind to TLR4 and AGER and stimulate intracellular signaling. High mobility group box 1 (HMGB1), S100B, and S100 calcium-binding protein B or A12 (S100A12) are well-known examples of proteins involved in DAMPs.

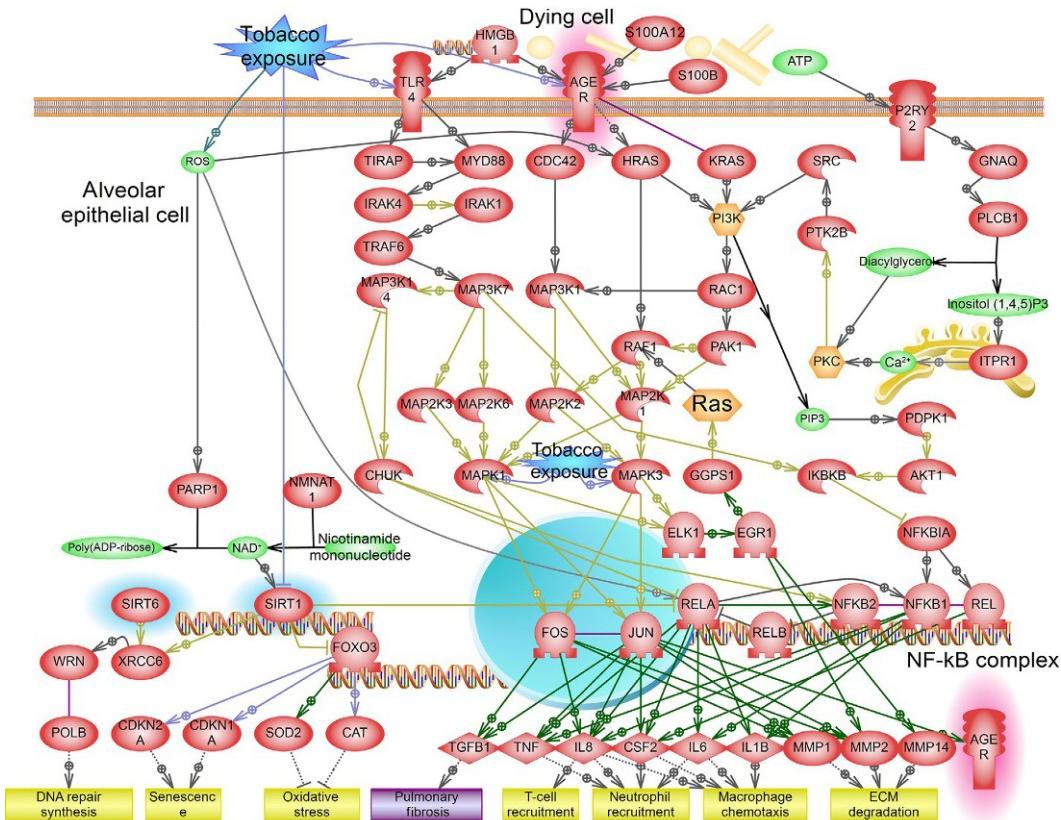
TLR4 and AGER signaling activates powerful transcription factors such as the JUN/FOS complex or NF- $\kappa$ B and in turn stimulate expression of a wide range of proteins. Activation of the transcription factor early growth response 1 (EGR1) sends the signal back to the MAPK1/MAPK3 pathway via activation of RAF1, forming a vicious circle. EGR1 also can

control expression of the matrix metalloproteinases MMP2 and MMP14. High levels of metalloproteinases cause the destruction of alveolar septal architecture.

High levels of extracellular ATP (which is also considered a component of a DAMP) cause the release of the proinflammatory cytokines IL-8 and IL-6 via activation of purinergic receptor P2Y2 (P2RY2) signaling in alveolar epithelial cells.

Sirtuins are deacetylases with a wide array of targets in the nucleus and cytoplasm. Properly activated SIRT1 plays a protective role in the cell stress response by decreasing gene transcription, inhibiting cell proliferation, and reducing oxidative stress. Sirtuins require NAD<sup>+</sup> for their activation. The levels of NAD<sup>+</sup> change during oxidative stress or inflammation. The activity of sirtuins (SIRT1 and SIRT6) was observed to be decreased in patients with COPD in response to cigarette smoke exposure, probably due to posttranslational oxidative modification by cigarette smoke-derived components. The reduction of SIRT1 and SIRT6 activities cause elevated cytokine production through NF- $\kappa$ B activation. It is also involved in regulating the expression of the cell cycle regulators (CDKN1A and CDKN2A) and activation of DNA repair-related proteins ([Barnes, 2013](#); [Brusselle et al., 2011](#); [Geraghty et al., 2011](#); [Ito and Barnes, 2009](#); [MacNee, 2009](#); [Rahman et al., 2012](#); [Sarir et al., 2008](#); [Shen et al., 2011](#); [Sundar et al., 2013](#)).

## II. Human disease pathways



**FIG. 9** Pathway 1: Cigarette smoke causes dysfunction and production of inflammatory mediators in alveolar epithelial cells.

## Pathway 2

### Cigarette smoke and inflammation cause alveolar epithelial cell death in COPD (Fig. 10)

#### Incoming signals

Cigarette smoke, air pollutants, and inflammation may induce self-autophagy and apoptosis of alveolar epithelial cells.

#### Outcome effects

Loss of alveolar epithelial cells either by apoptosis or by self-autophagy is one of the significant factors in the development of pulmonary emphysema (i.e., the loss of lung parenchyma with the concomitant enlargement of air spaces), a prominent hallmark of COPD.

#### Signaling

Several different signaling mechanisms may induce apoptosis in response to harmful extracellular signals. The Fas cell surface death receptor (FAS) and tumor necrosis factor receptor (TNFRSF1A) are major receptors that trigger the apoptosis signaling pathway. These receptors can be stimulated by the Fas ligand (FASLG) and by tumor necrosis factor (TNF), which is either produced by alveolar epithelial cells or recruited to the lungs by leukocytes involved in the inflammatory reaction. The FAS receptor stimulates apoptosis through the formation of the death-inducing signal complex with Fas-associated via death domain (FADD) and caspase 8 (CASP8). Following cleavage, mature CASP8 activates CASP3 or cleaves the mitochondrial protein BH3 interacting domain death agonist (BID). Truncated BID interacts with mitochondrial BCL2-associated X protein (BAX) resulting in the release of cytochrome *c* (CYCS) through a pore in the outer mitochondrial membrane. This also contributes to CASP3 activation. CASP3 cleaves DNA fragmentation factor subunit alpha (DFFA) and releases it from DFFB, which is then translocated to the nucleus and acts as an active DNA endonuclease.

Cigarette smoke may induce apoptosis in alveolar epithelial cell because of aggressive autophagy and an enhanced endoplasmic reticulum (ER) stress response. If the ER stress response is initiated, eukaryotic translation initiating factor 2-alpha kinase 3 (EIF2AK3) and the transcription factors ATF4 and ATF6 mediate the induction of DNA damage-inducible transcript 3 (DDIT3) expression, which initiates autophagy and also leads to mitochondrial-dependent apoptosis. Autophagy of cytoplasmic components (macroautophagy) is promoted by activated autophagic proteins

such as autophagy-related 4B, 5, 12 (ATG4B, ATG5, and ATG12) as well by the dissociation of beclin 1 (BECN1) from the B-cell lymphoma protein 2 alpha (BCL2) or BCL2L1. The MAP1LC3B/CAV1/FAS multimeric complex may mediate the effects of cigarette smoke on the initiation of autophagy in COPD. Although the exact role of microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) in the alveolar epithelial cell is not clear.

Also, CASP12 is activated in response to endoplasmic reticulum (ER) stress and other stress signals such as hypoxia in COPD alveolar epithelial cell.

Finally, cytotoxic T cells, which are attracted to the site of inflammation, release the pore-forming proteins granzyme B (GZMB) and perforin (PRF1) that induce apoptosis in alveolar epithelial cells (Adair-Kirk et al., 2008; Chen et al., 2010; Demedts et al., 2006; Kang et al., 2011; Kelsen et al., 2008; Morissette et al., 2009; Ribeiro and O'Neal, 2012; Rovina et al., 2013; Ryter et al., 2009, 2010, 2011; Ryter and Choi, 2010; Zhou et al., 2011).

## II. Human disease pathways

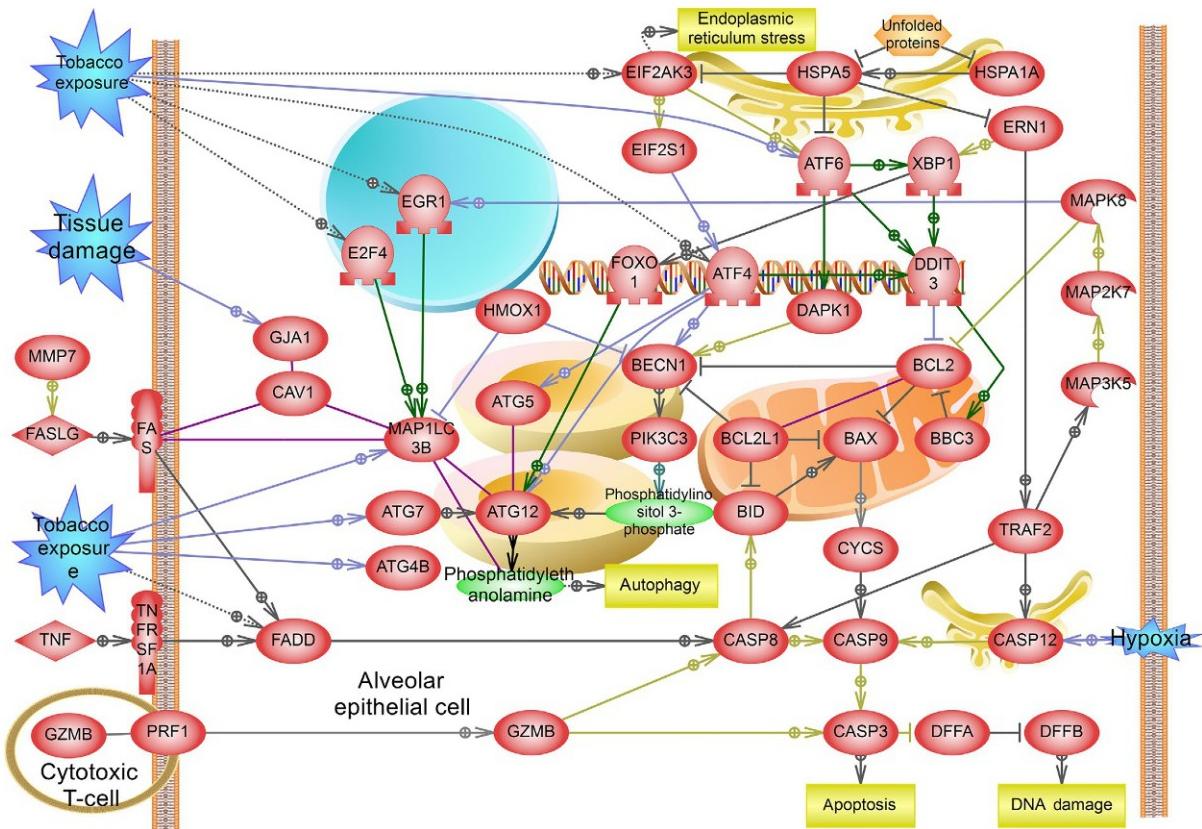


FIG. 10 Pathway 2: Cigarette smoke and inflammation cause alveolar epithelial cell death in COPD.

## Pathway 3

### Role of alveolar macrophages in pulmonary tissue destruction in COPD ([Fig. 11](#))

#### Incoming signals

Alveolar macrophages are believed to play a pivotal role in the pathophysiology of COPD. Alveolar macrophages localize at the boundary between the lungs and external environment. Macrophages are responsible for the removal of particles, such as dust or microorganisms, from the respiratory surfaces by phagocytosis. To be effective, they must maintain a high level of activity.

Cigarette smoke together with bacterial and viral infections causes hyperactivation of innate immune cells, including macrophages, by triggering pattern recognition receptors (PRRs) such as the toll-like receptors (TLRs). TLR signaling induces the expression of proinflammatory proteins and activates the phagocytosis of surrounding cells by macrophages, leading to lung tissue damage.

#### Outcome effects

Chronic lung inflammation and tissue remodeling by proteolytic enzymes are the consequence of sustained macrophage activation in COPD ([Hussell and Bell, 2014](#)).

#### Signaling

Alveolar epithelial cells, activated during inflammation and stress, secrete a variety of inflammatory mediators and chemokines, which can attract macrophages (see [Pathway 1](#)).

Dying alveolar epithelial cells release several DAMPs include HMGB1, uric acid, extracellular ATP, heat shock proteins, hyaluronic acid, versican (VCAN), and heparan sulfate. DAMP, as well as bacterially, derived lipopolysaccharides (LPS), bind to TLR2, TLR4, and other receptors on the macrophage surface to stimulate their function.

TLRs activate myeloid differentiation primary response 88 (MYD88), toll-like receptor 4 adaptor protein (TIRAP), and TICAM1/2 signaling, which leads to the synthesis of inflammatory cytokines (IL-6, IL-8, IL-1A, IL-1B, and IL-18), chemokines (CXCL1 and CCL2), matrix metalloproteinases (MMP2, MMP9, and MMP12), and lysosomal cysteine proteinases (CTSL1 and CTSS).

Inflammatory proteins secreted by other injured cells also bind to and stimulate other receptors such as AGER or IL-1R1on macrophages.

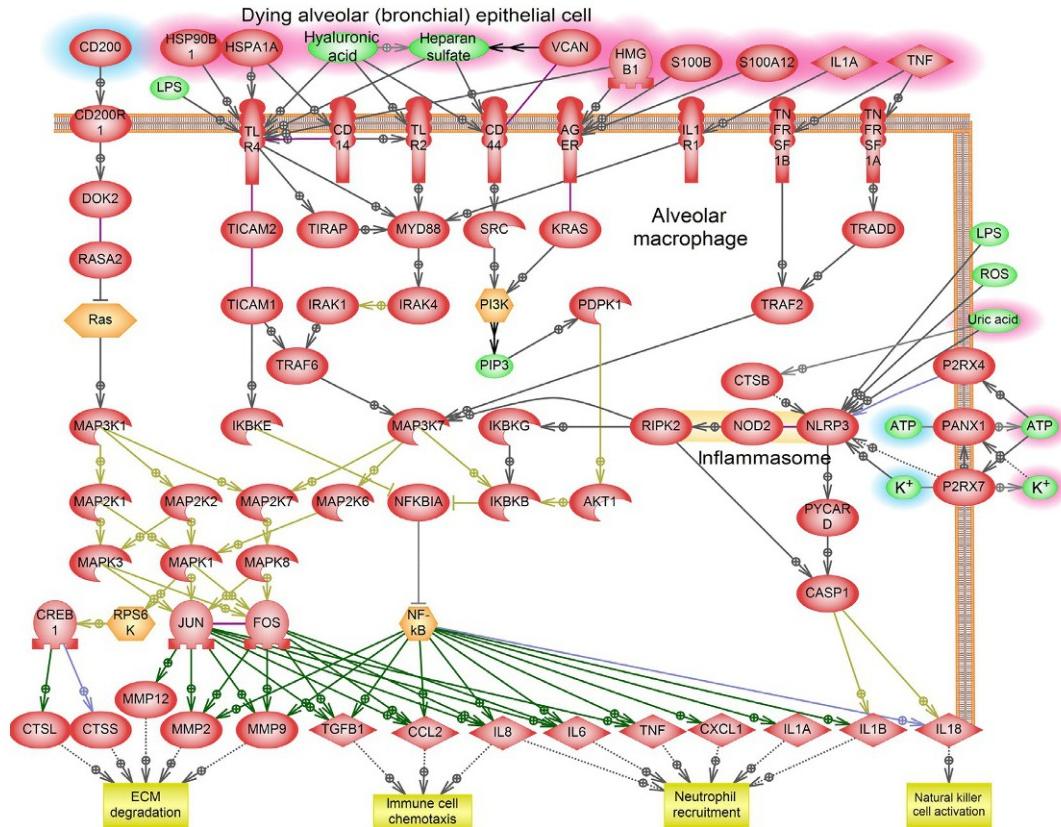
Expression of the most of inflammatory proteins that are upregulated in COPD macrophages is dependent on NF- $\kappa$ B transcription factor activation.

The NLRP3 inflammasome is another pathogen and damage-associated molecular pattern (DAMP) sensor, which may be activated by high levels of ATP, ROS, uric acid, and bacterial LPS. NLR family, pyrin domain-containing 3 (NLRP3) inflammasome assembly also may be triggered by low intracellular potassium ( $K^+$ ) levels. The inflammasome activates CASP1, triggering apoptosis and subsequently cleaves pro-IL-1B and pro-IL-18 into mature interleukins.

Most of the inflammatory mediators, pathogens, and molecules from apoptotic cells are potent activators of phagocytosis. This part of the signaling network is not shown here, but it involves the activation of CD14, MSR1, and MCR1 receptors expressed on the macrophage plasma membrane.

Usually, after the completion of the function of alveolar macrophages, they are negatively regulated, mainly by CD200 (thymocyte antigen identified by monoclonal antibody MRC-OX2). CD200 is present on bronchial and alveolar epithelial cells. It couples with the macrophage CD200 receptor (CD200R1). CD200R1 recruits are docking protein 2 (DOK2) and protein RAS p21 protein activator II (RASA2), which together inhibit the expression of proinflammatory proteins by the JUN-FOS transcription complex. In COPD and pulmonary emphysema, the suppression of macrophages by CD200 is impaired due to the death of bronchial and alveolar epithelial cells (Brusselle et al., 2011; Cicko et al., 2010; Doz et al., 2008; Mortaz et al., 2009, 2010; Rovina et al., 2013; Sarir et al., 2008).

## II. Human disease pathways



**FIG. 11** Pathway 3: Role of alveolar macrophages in pulmonary tissue destruction in COPD.

## Pathway 4

### Mucin hypersecretion in COPD (Fig. 12)

#### Incoming signals

Patients with COPD, similarly to patients with asthma, have increased luminal mucus secretion (see Asthma). Mucin hypersecretion is strengthened especially with age and with chronic respiratory tract infections.

Mechanisms of mucus secretion in COPD are similar to those in asthma (see Asthma: Pathway 3), but there are significant differences. In COPD the mucus is less viscous so that mucins are easier to remove from the airways. The ratio of the mucins MUC5AC/MUC5B may also be reduced. Also in COPD, there is no noticeable fluid exudation into the airways ([Lai and Rogers, 2010](#); [Rogers, 2000](#)). There is a hypothesis that the proteases released from neutrophils, which themselves predominate in the airways of patients with COPD, can cleave mucins attached to the cell surface of goblet cells. Eosinophils, which predominate in asthmatic airways, do not contribute to the cleaving of mucins, so mucus forms tenacious plugs in asthma ([Garcia-Verdugo et al., 2010](#); [Sutherland and Martin, 2003](#)).

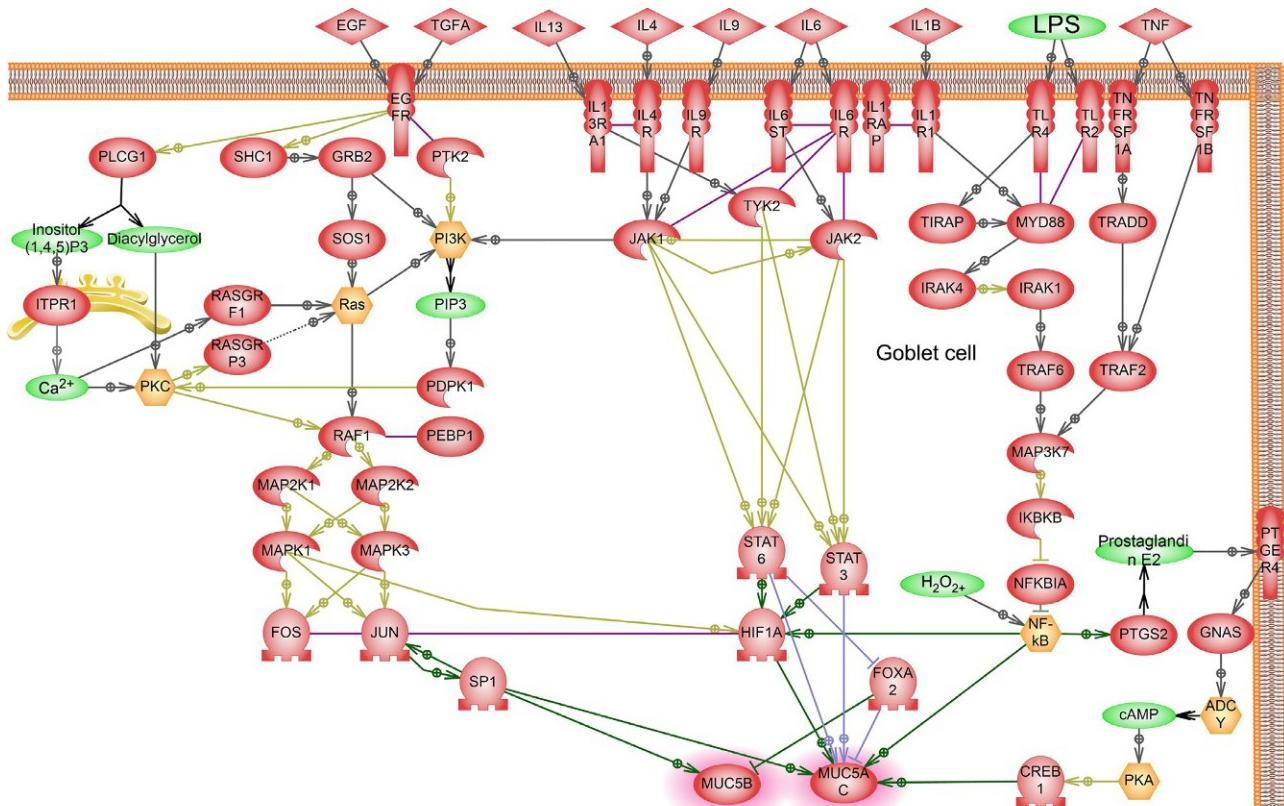
#### Outcome effects

Excessive mucus production and impaired mucociliary clearance cause airway obstruction and facilitate the spread of respiratory infections in patients with COPD ([Fahy and Dickey, 2010](#); [Voynow and Rubin, 2009](#)).

#### Signaling

For the description of relevant signaling, see "Asthma: Pathway 3. Airway Surface Liquid (ASL) thickness and Mucus accumulation in Asthma."

## II. Human disease pathways



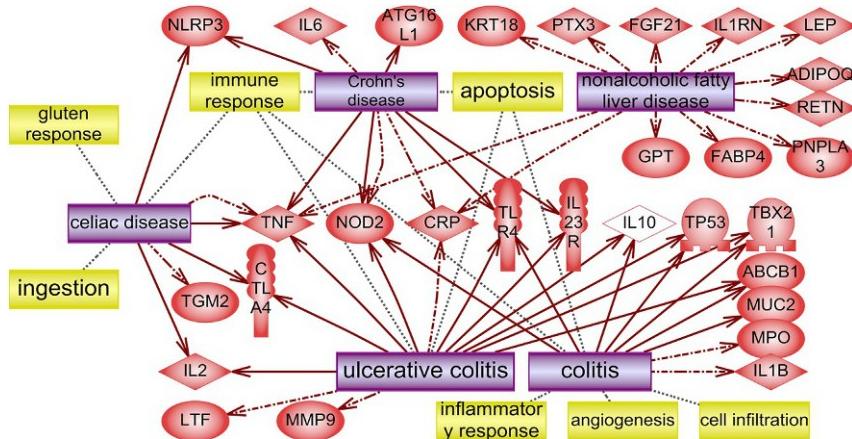
**FIG. 12** Pathway 4: Mucin hyperproduction in goblet and mucous cells in COPD.

## References

- Disease number #606963 and #130700 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code J44. Diseases of the respiratory system (J00-J99). (ICD-10, <https://icdlist.com>). ICD-11: disease code CA22.
- Adair-Kirk, T.L., Atkinson, J.J., Senior, R.M., 2008. Smoke particulates stress lung cells. *Nat. Med.* 14, 1024–1025. <https://doi.org/10.1038/nm1008-1024>.
- Barnes, P.J., 2013. New anti-inflammatory targets for chronic obstructive pulmonary disease. *Nat. Rev. Drug Discov.* 12, 543–559.
- Brusselle, G.G., Joos, G.F., Bracke, K.R., 2011. New insights into the immunology of chronic obstructive pulmonary disease. *Lancet* 378, 1015–1026. [https://doi.org/10.1016/S0140-6736\(11\)60988-4](https://doi.org/10.1016/S0140-6736(11)60988-4).
- Chen, Z.-H., Lam, H.C., Jin, Y., Kim, H.-P., Cao, J., Lee, S.-J., Ifedigbo, E., Parameswaran, H., Ryter, S.W., Choi, A.M.K., 2010. Autophagy protein microtubule-associated protein 1 light chain-3B (LC3B) activates extrinsic apoptosis during cigarette smoke-induced emphysema. *Proc. Natl. Acad. Sci. U. S. A.* 107, 18880–18885. <https://doi.org/10.1073/pnas.1005574107>.
- Cicko, S., Lucattelli, M., Müller, T., Lommatsch, M., De Cunto, G., Cardini, S., Sundas, W., Grimm, M., Zeiser, R., Dürk, T., Zissel, G., Boeynaems, J.-M., Sorichter, S., Ferrari, D., Di Virgilio, F., Virchow, J.C., Lungarella, G., Idzko, M., 2010. Purinergic receptor inhibition prevents the development of smoke-induced lung injury and emphysema. *J. Immunol.* 185, 688–697. <https://doi.org/10.4049/jimmunol.0904042>.
- Demedts, I.K., Demoor, T., Bracke, K.R., Joos, G.F., Brusselle, G.G., 2006. Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir. Res.* 7, 53. <https://doi.org/10.1186/1465-9921-7-53>.
- Doz, E., Noulin, N., Boichot, E., Guénon, I., Fick, L., Le Bert, M., Lagente, V., Ryffel, B., Schnyder, B., Quesniaux, V.F.J., Couillin, I., 2008. Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. *J. Immunol.* 180, 1169–1178.
- Fahy, J.V., Dickey, B.F., 2010. Airway mucus function and dysfunction. *N. Engl. J. Med.* 363, 2233–2247. <https://doi.org/10.1056/NEJMra0910061>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Garcia-Verdugo, I., Descamps, D., Chignard, M., Touqui, L., Sallenave, J.-M., 2010. Lung protease/anti-protease network and modulation of mucus production and surfactant activity. *Biochimie* 92, 1608–1617. <https://doi.org/10.1016/j.biochi.2010.05.010>.
- Geraghty, P., Dabo, A.J., D'Armiento, J., 2011. TLR4 protein contributes to cigarette smoke-induced matrix metalloproteinase-1 (MMP-1) expression in chronic obstructive pulmonary disease. *J. Biol. Chem.* 286, 30211–30218. <https://doi.org/10.1074/jbc.M111.238824>.
- Hussell, T., Bell, T.J., 2014. Alveolar macrophages: plasticity in a tissue-specific context. *Nat. Rev. Immunol.* 14, 81–93. <https://doi.org/10.1038/nri3600>.
- Ito, K., Barnes, P.J., 2009. COPD as a disease of accelerated lung aging. *Chest* 135, 173–180. <https://doi.org/10.1378/chest.08-1419>.
- Kang, R., Zeh, H.J., Lotze, M.T., Tang, D., 2011. The Beclin 1 network regulates autophagy and apoptosis. *Cell Death Differ.* 18, 571–580. <https://doi.org/10.1038/cdd.2010.191>.
- Kelsen, S.G., Duan, X., Ji, R., Perez, O., Liu, C., Merali, S., 2008. Cigarette smoke induces an unfolded protein response in the human lung: a proteomic approach. *Am. J. Respir. Cell Mol. Biol.* 38, 541–550. <https://doi.org/10.1165/rcmb.2007-0221OC>.
- Lai, H., Rogers, D.F., 2010. New pharmacotherapy for airway mucus hypersecretion in asthma and COPD: targeting intracellular signaling pathways. *J. Aerosol Med. Pulm. Drug Deliv.* 23, 219–231. <https://doi.org/10.1089/jamp.2009.0802>.

- MacNee, W., 2009. Accelerated lung aging: a novel pathogenic mechanism of chronic obstructive pulmonary disease (COPD). *Biochem. Soc. Trans.* 37, 819–823. <https://doi.org/10.1042/BST0370819>.
- Morissette, M.C., Parent, J., Milot, J., 2009. Alveolar epithelial and endothelial cell apoptosis in emphysema: what we know and what we need to know. *Int. J. Chron. Obstruct. Pulmon. Dis.* 4, 19–31.
- Mortaz, E., Braber, S., Nazary, M., Givi, M.E., Nijkamp, F.P., Folkerts, G., 2009. ATP in the pathogenesis of lung emphysema. *Eur. J. Pharmacol.* 619, 92–96. <https://doi.org/10.1016/j.ejphar.2009.07.022>.
- Mortaz, E., Folkerts, G., Nijkamp, F.P., Henricks, P.A.J., 2010. ATP and the pathogenesis of COPD. *Eur. J. Pharmacol.* 638, 1–4. <https://doi.org/10.1016/j.ejphar.2010.04.019>.
- Rahman, I., Kinnula, V.L., Gorbulova, V., Yao, H., 2012. SIRT1 as a therapeutic target in inflammmaging of the pulmonary disease. *Prev. Med.* 54 (Suppl), S20–S28. <https://doi.org/10.1016/j.ypmed.2011.11.014>.
- Ribeiro, C.M.P., O'Neal, W.K., 2012. Endoplasmic reticulum stress in chronic obstructive lung diseases. *Curr. Mol. Med.* 12, 872–882.
- Rogers, D.F., 2000. Mucus pathophysiology in COPD: differences to asthma, and pharmacotherapy. *Monaldi Arch. Chest Dis. Arch. Monaldi Mal. Torace Fondazione Clin. Lav. IRCCS Ist. Clin. Tisiol. E Mal. Appar. Respir. Univ. Napoli Secondo Ateneo* 55, 324–332.
- Rovina, N., Koutsoukou, A., Koulouris, N.G., 2013. Inflammation and immune response in COPD: where do we stand? *Mediat. Inflamm.* 2013, 413735. <https://doi.org/10.1155/2013/413735>.
- Ryter, S.W., Choi, A.M.K., 2010. Autophagy in the lung. *Proc. Am. Thorac. Soc.* 7, 13–21. <https://doi.org/10.1513/pats.200909-101JS>.
- Ryter, S.W., Chen, Z.-H., Kim, H.P., Choi, A.M.K., 2009. Autophagy in chronic obstructive pulmonary disease: homeostatic or pathogenic mechanism? *Autophagy* 5, 235–237.
- Ryter, S.W., Lee, S.-J., Choi, A.M., 2010. Autophagy in cigarette smoke-induced chronic obstructive pulmonary disease. *Expert Rev. Respir. Med.* 4, 573–584. <https://doi.org/10.1586/ers.10.61>.
- Ryter, S.W., Lam, H.C., Chen, Z.-H., Choi, A.M.K., 2011. Deadly triplex: smoke, autophagy and apoptosis. *Autophagy* 7, 436–437.
- Sarir, H., Henricks, P.A.J., van Houwelingen, A.H., Nijkamp, F.P., Folkerts, G., 2008. Cells, mediators and Toll-like receptors in COPD. *Eur. J. Pharmacol.* 585, 346–353. <https://doi.org/10.1016/j.ejphar.2008.03.009>.
- Shen, N., Gong, T., Wang, J.-D., Meng, F.-L., Qiao, L., Yang, R.-L., Xue, B., Pan, F.-Y., Zhou, X.-J., Chen, H.-Q., Ning, W., Li, C.-J., 2011. Cigarette smoke-induced pulmonary inflammatory responses are mediated by EGR-1/GGPPS/MAPK signaling. *Am. J. Pathol.* 178, 110–118. <https://doi.org/10.1016/j.ajpath.2010.11.016>.
- Sundar, I.K., Yao, H., Rahman, I., 2013. Oxidative stress and chromatin remodeling in chronic obstructive pulmonary disease and smoking-related diseases. *Antioxid. Redox Signal.* 18, 1956–1971. <https://doi.org/10.1089/ars.2012.4863>.
- Sutherland, E.R., Martin, R.J., 2003. Airway inflammation in chronic obstructive pulmonary disease: comparisons with asthma. *J. Allergy Clin. Immunol.* 112, 819–827. [https://doi.org/10.1016/S0091-6749\(03\)02011-6](https://doi.org/10.1016/S0091-6749(03)02011-6).
- Voynow, J.A., Rubin, B.K., 2009. Mucins, mucus, and sputum. *Chest* 135, 505–512. <https://doi.org/10.1378/chest.08-0412>.
- Zhou, F., Yang, Y., Xing, D., 2011. Bcl-2 and Bcl-xL play important roles in the cross-talk between autophagy and apoptosis. *FEBS J.* 278, 403–413. <https://doi.org/10.1111/j.1742-4658.2010.07965.x>.

# Diseases of the digestive system



## OUTLINE

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Diseases of the digestive system cover dysfunctions of the organs of the gastrointestinal tract including the esophagus, stomach, liver, gallbladder, pancreas, and large and small intestines. Infections, allergic reactions, inflammation, and genetic predispositions are the major causes of intestinal diseases. Symptoms of diseases of the gastrointestinal tract are very diverse and often include diarrhea, nausea and vomiting, bloating, pain in

the abdomen, and weight gain or weight loss. Most common disorders of the digestive system include food poisoning, gastroesophageal reflux disease, constipation, and similar indigestion-related problems. Even though there are common acute pathological conditions of the digestive tract, they may become chronic and need lifelong management. Both genetic predispositions and environmental factors are important factors in the pathogenesis of gastrointestinal disorders; the resulting functional insufficiency of the digestive system can be controlled by changes in lifestyle. This chapter describes the most common complex and severe diseases of the bowel—Crohn's disease, ulcerative colitis, and celiac disease. Chronic immune and inflammatory responses are the most common pathological mechanisms underlying these conditions. There are many similarities between the mechanisms of molecular pathogenesis for Crohn's disease and ulcerative colitis. It is difficult to make an accurate diagnosis, distinguish among the triggers, and determine the relevant genetic bases for the predisposition. However, unlike Crohn's disease, ulcerative colitis affects only the large intestine and causes the formation of sores or ulcers.

Celiac disease is a result of an autoimmune reaction to the plant protein gluten found in wheat, rye, and barley. A genetic predisposition is a primary factor in the progression of the disease. People with celiac disease may not have distinctive symptoms, or they may have symptoms that mimic other immunological conditions. There is a concern that celiac disease remains undiagnosed in many cases, so its prevalence may be much broader than previously thought.

Nonalcoholic fatty liver disease (NAFLD) is a representative liver disorder detailed in the chapter. NAFLD is considered a common liver disease whose progression is linked to increased fat deposition or steatosis. In NAFLD the characteristic inflammation and damage to the fatty liver are similar to the damage caused by heavy alcohol use. The rapid spread of diabetes and obesity in modern societies has a positive influence on the frequency of NAFLD occurrence.

## CHAPTER

## 10.1

## Crohn's disease

Crohn's disease is a complex, chronic disorder that primarily affects the digestive system. This condition associated with strong inflammation in the lower part of the small intestine (the ileum) and portions of the large intestine (the colon). Inflammation of the intestinal walls can occur in any part of the digestive system, however. Studies suggest that Crohn's disease may result from a combination of certain genetic variations, changes in the immune system, and the presence of bacteria in the digestive tract (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Crohn's disease is an inflammatory disease of the bowel of unknown etiology, most commonly involving the terminal ileum and manifesting primarily with diarrhea, abdominal pain, fatigue, and weight loss. (*Ferri, 2017, p. 1*).

Crohn's disease is a chronic autoimmune granulomatous inflammation of the gastrointestinal tract. (*Marks et al., 2010*).

An inflammatory phenomenon of the Crohn's disease associated with an adaptive T-cell response in addition to an abnormal function of the innate immune system. (*Tozer et al., 2009*).

Crohn's disease is a complex disease that presents with several clinical forms. The existence of two distinct forms has been suggested: perforating and nonperforating, where perforating is a more aggressive one.

A genetic predisposition is undoubtedly important for the development of Crohn's disease. Understanding the puzzle of Crohn's disease genetics is complicated by the genetic heterogeneity of the disease. Several analyses have demonstrated the complex genetic basis of Crohn's disease. For example, there is clear evidence that mutations in genes located on the locus on chromosome 16 increase the risk of developing Crohn's disease (*Cavanaugh and IBD International Genetics Consortium, 2001*). Polymorphisms of the nucleotide-binding oligomerization domain containing 2 (NOD2) gene from that locus were identified as one of the main contributors for both inflammatory bowel disease and Crohn's disease susceptibility (*van Heel et al., 2004*). The contribution of polymorphisms in other genes was also shown. To study the inheritance of Crohn's disease, it is important to consider that various combinations of several mutated genes interaction lead to similar clinical symptoms.

There is a significant overlap between the genetic factors involved in Crohn's disease and ulcerative colitis. At the same time, significant differences were found in the expression patterns of genes and in polymorphisms associated with either ulcerative colitis or Crohn's disease (see ulcerative colitis).

Triggered by an inflammatory reaction and supported by the host microbiome, hyperexpression of proinflammatory proteins by dendritic cells, macrophages and epithelial cells lead to the abnormally strong activation of T-helper cell types 1 and 17 (Th1-cells and Th17-cells) in the intestinal walls. Variations in several genes, including autophagy-related 16 like 1 (ATG16L1) and NOD2, contribute to the inability of intestinal cells to provide an epithelial and mucosal barrier against commensal microflora present in the gut:

**Pathway 1.** *Defects in response to pathogens in the gut promotes inflammation in Crohn's disease* ([Fig. 1](#)).

Failed autophagy and a decline in the synthesis of defensins by antigen-presenting cells and Paneth cells are thought to lead to high levels of bacterial penetration to intestine tissue. Entry of pathogenic organisms or their component parts into the lamina propria and the migration of antigen-presenting cells to Peyer's patches with antigens then stimulate the T-helper cell response and Crohn's-like inflammation in the intestine:

**Pathway 2.** *Paneth cell dysfunction in Crohn's disease* ([Fig. 2](#)).

**Pathway 3.** *Autophagy dysfunction in Crohn's disease* ([Fig. 3](#)).

## Key cellular contributors and processes

Antigen-presenting cells

Cell

Antigen-presenting cells (APCs) are a large group of various cells that trigger the cellular immune response by processing an antigen and exposing it in a form recognizable by T cells in the process known as antigen presentation.

Autophagy

Process

Autophagy is a conserved eukaryotic process in which excessive or dysfunctional intracellular components are delivered to lysosomes for degradation. The three major types of autophagy include macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membrane vesicle, the autophagosome.

Inflammasome

Anatomic structure

The inflammasome is a multiprotein complex of the innate immune response system. The inflammasome activates the expression of proinflammatory interleukins 1 $\beta$  (IL-1 $\beta$ ) and 18 (IL-18) and promotes inflammation. Dysregulation of inflammasome function is involved in the pathogenesis in a variety of autoimmune diseases.

NOD-like receptors

Protein

The NOD-like receptors (nucleotide-binding oligomerization domain-like receptors, NLRs) are cytoplasmic pattern recognition receptors. NLRs can bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) inside the cell and have a variety of functions in the regulation of inflammatory and apoptotic responses. The NLR family consists of several proteins divided into subfamilies based on their N-terminal protein-interacting domains.

Paneth cell

Cell

Paneth cells are specialized secretory epithelial cells of the small intestine that produce antimicrobial peptides and are key players in the intestinal innate immune defense.

## Pathogen pattern recognition

### Process

During the initial stage of response to infection, the innate defense system employs pattern recognition receptors (PRRs) that recognize components of invading pathogens. The PRRs can detect evolutionarily conserved molecular structures called pathogen-associated molecular patterns (PAMPs) derived from bacteria and viruses and initiate an antimicrobial inflammatory response. At the same time, PRRS can also detect damage-associated molecular patterns (DAMPs) released from host cells during cell damage or death and initiate a noninfectious inflammatory response.

## Peyer's patches

### Anatomic structure

Peyer's patches (PPs) are organized lymphoid nodules in the mucous layer of the ileum (a segment in the small intestine). PP appear as round or oval aggregates located in the epithelial mucosa membrane lining and have a primary role in the induction of mucosal immunity in the gut.

## Pleiotropic gene

### Protein or gene

Pleiotropic gene is one that when mutated produces several markedly different phenotypic traits.

## Proinflammatory cytokines

### Process

Cytokines are a broad list of small proteins released by immune cells, which participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony stimulating factor (GMCS-F), and others), secreted primarily by macrophages and T-helper cells, upregulate proinflammatory reactions.

## Th17 cells

### Cell

Th17 cells are a subset of T-helper lineage cells that preferentially express interleukin 17A, 17F, 21, and 22. The Th17 cells act as proinflammatory agents by recruiting neutrophils and macrophages to the infected site. Th17 cells are implicated in the development of autoimmune diseases.

## Type 1 and Type 2 T-helper cells

### Cell

Type 1 and Type 2 T-helper cell (Th1, Th2 cell) are cells of the T-cell lineage that protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) via stimulation of B-cell maturation and activation of other immune cells.

## Pathway 1

### Defects in response to pathogens in the gut promotes inflammation in Crohn's disease ([Fig. 1](#))

#### Incoming signals

The altered function of proteins involved in pathogen recognition in antigen-presenting cells of the intestinal tract of patients with Crohn's disease is thought to be the reason for an increased response of the gut to microbes, which leads to a chronic inflammatory reaction. There is a notion that *Mycobacterium paratuberculosis* could be the primary pathogen responsible for triggering Crohn's disease.

Impaired genetic control of the intestinal barrier has long been suspected to be a predisposing factor for Crohn's disease. Mutations in one of the pathogen pattern recognition genes (specifically the *NOD2* gene) were found to have strong associations with Crohn's disease in several human populations, excluding the Japanese ([Yamazaki et al., 2002](#)). *NOD2* is a pleiotropic gene that confers susceptibility not only to Crohn's disease but also to ulcerative colitis, Blau syndrome, and psoriatic arthritis. The predominant mutations in *NOD2* that are associated with Crohn's disease in several populations are found in the SNP8 (R702W), SNP12 (G908R), and SNP13 (1007fs) genes. Different mutations in *NOD2* lead to different manifestations of the disease, and there may be a gene-dosage effect as well. *NOD2* regulates the cellular response to pathogens by means of enhanced inflammasome activation (1) nuclear factor kappaB (NF- $\kappa$ B) related proinflammatory cytokine expression (2) and autophagy control (see [Pathway 3](#)).

Despite the strong association of *NOD2* genes with Crohn's disease, it is considered a genetically heterogeneous disease. For example, variants in other genes of the same locus on chromosome 16q were also found to be associated with Crohn's disease. They include *CYLD* (CYLD lysine 63 deubiquitinase), interferon regulatory factor 8 (IRF8) region, and the region containing *CDH1* and *CDH3* (cadherin 1,3).

TNF superfamily member 11 (TNFSF11) was also associated with an increased risk for Crohn's disease due to increased levels in the serum of patients with Crohn's disease. The origin and causes of the observed increased expression levels of TNFSF11 are not well understood. The high levels of expression of TNFSF11 in patients with Crohn's disease facilitate antigen-presenting cells migrating to Peyer's patches (intestinal mesenteric lymph nodes) to present antigens and in turn stimulate T-cell proliferation and differentiation ([Franchimont et al., 2004](#)).

## Outcome effects

The experimental data regarding the exact level of proinflammatory cytokines in Crohn's disease are controversial, probably due to the complex nature of the disease. Increased expression levels of interleukins IL-12B, IL-18, IL-18BP, IL-1B, and IL-1RA were detected in some intestinal cells from patients with Crohn's disease.

Cytokines released from antigen-presenting cells activate themselves along with Th1 and Th17 cells. Intestinal Th17 cells react more intensely in patients with Crohn's disease and demonstrate constitutive activation of the signal transducer and activator of transcription 3-4 (STAT3-4) and major transcription factors responsible for T-cell function ([Lovato et al., 2003](#)). Normally the proinflammatory activity of Th17 cells can be beneficial to the host during infection. However, uncontrolled Th17 activation leads to autoimmune and autoinflammatory damage of intestinal tissues. Anti-IL-12 and Anti-IL-23 antibody therapy, which targets both Th1 cells and Th17 cells, is an effective treatment for Crohn's disease.

Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) that overexpressed in the ileal mucosa in Crohn's disease also contributes to bacteria penetration into the intestine tissue ([Barnich et al., 2007](#)). The activation of CEACAM6 expression was shown to be stimulated by high levels of inflammatory cytokines such as TNF. CEACAM6 acts on the apical surface of ileal epithelial cells binding with the surface of *Escherichia coli*.

## Signaling

NOD2 is an intracellular receptor expressed in a variety of immune and nonimmune cells where it acts as a general sensor of pathogens. NOD2 belongs to the family of intracellular NOD-like receptors that contain a CARD domain (caspase-recruiting domain). Both NOD1 and NOD2 recognize peptidoglycan (PGN)-related molecules synthesized in bacteria. NOD1 recognizes the D-gamma-glutamyl-meso-diaminopimelic acid dipeptide (iE-DAP), which is found in the PGN of Gram-negative bacteria, while NOD2 recognizes the muramyl dipeptide (MDP) found in almost all bacteria.

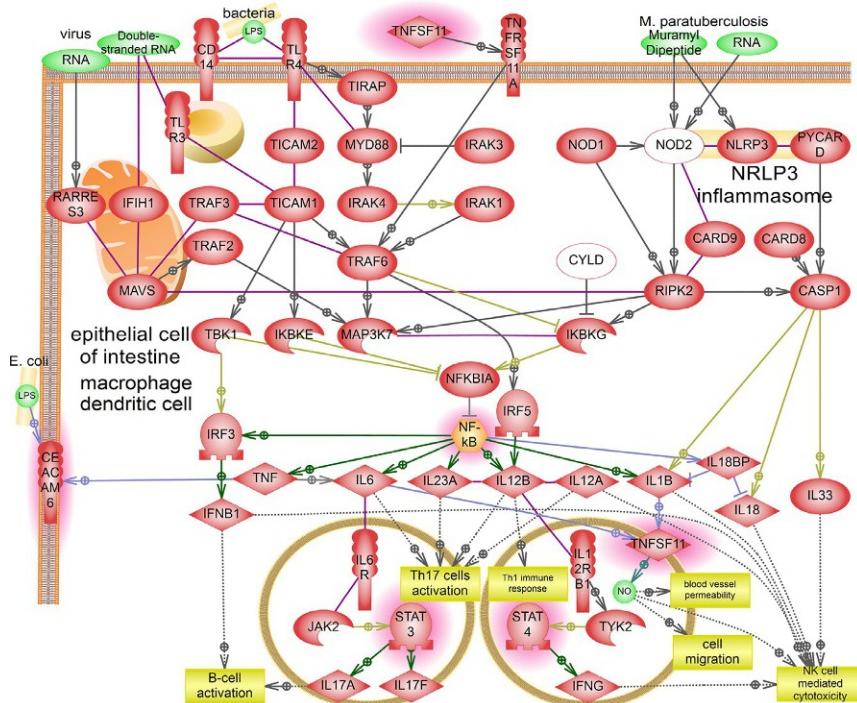
- (1) The inflammasome is a multiprotein complex formed by NOD2 with NLR receptors (e.g., NLRP1 and NLR family pyrin domain containing 1,3 (NLRP3)) and adaptor proteins (such as caspase recruitment domain family member 8 (CARD8)). Caspase 1 (CASP1) activation in inflammasomes leads to the maturation and activation

of interleukin 1B (IL-1B), interleukin 18 (IL-18), and interleukin 33 (IL-33). These cytokines in turn activate macrophages and T cells. NLRP3 expression in inflammasomes can also be induced by a wide variety of stimuli including whole bacteria (e.g., *Listeria monocytogenes* and *Staphylococcus aureus*), bacterial RNA, uric acid crystals, the amyloid beta protein, extracellular ATP, and pore-forming toxins (e.g., nigericin and maitotoxin).

- (2) NF- $\kappa$ B related proinflammatory cytokine expression was shown to rise in cells carrying a mutated NOD2 gene. Activated NOD2 binds to receptor-interacting serine/threonine kinase 2 (RIPK2) that mediates the ubiquitination of inhibitor of nuclear factor kappa B kinase subunit gamma (IKBKG) and the recruitment of mitogen-activated protein kinase kinase kinase 7 (MAP3K7) leading to the activation of NF- $\kappa$ B. Also, NOD2 interacts with the adapter protein, caspase recruitment domain family member 9 (CARD9), to mediate MAPK3K7 and MAPK8 signaling (not shown) through interaction with receptor-interacting serine/threonine kinase 2 (RIPK2). NOD2 signaling intersects with standard toll-like receptor 4 (TLR4) signaling in antigen-presenting cells to stimulate the expression of proinflammatory cytokines.

Moreover, NOD2 signaling may be involved in the antiviral inflammatory responses via the mitochondrial antiviral signaling protein (MAVS), an adaptor protein associated with mitochondria. Although the details of the role viral infection on Crohn's disease progression are not known, it may lead to the typical activation of interferon regulatory factor 3 (IRF3) and the induction of interferon expression (e.g., interferon beta 1 (IFNB1)).

NOD2-related autophagy and mucosal secretion dysfunction (see [Pathways 2 and 3](#)) also play important roles in the increased bacterial penetration characteristic of Crohn's disease ([Brain et al., 2013; Cantó et al., 2009; Festen and Weersma, 2014; Kramer et al., 2006; Niess, 2008; Pizarro et al., 1999; Strober et al., 2008](#)).



**FIG. 1** Pathway 1: Defects in response to pathogens in the gut promotes inflammation in Crohn's disease.

## Pathway 2

### Paneth cell dysfunction in Crohn's disease (Fig. 2)

#### Incoming signals

Paneth cells are granulated epithelial cells of small intestinal crypts that secrete mucus; antimicrobial peptides; and proteins, including alpha-defensins, lysozyme, and secretory phospholipase A2, in response to bacterial products. Defects in Paneth cell function are believed to be a cause of the weak intestinal barrier and chronic inflammation in the gastrointestinal tract seen in patients with Crohn's disease.

#### Outcome effects

The natural antimicrobial protein defensin alpha 5 (DEFA5) is overexpressed, and several mucins (MUC1, MUC2, and others) are downregulated in the ileum and colon of patients with Crohn's disease. Alterations in NOD2 and changes in TLR4 signaling may lead to a defective mucosal barrier. NF- $\kappa$ B and a NOD2 inflammasome can initiate the expression of inflammatory cytokines in Paneth cells and in antigen-presenting cells (see [Pathway 1](#)).

#### Signaling

Patients with Crohn's disease who have *NOD2* mutations manifest more severe defects in alpha-defensin production than generally observed in others with Crohn's disease; however, the detailed molecular mechanisms of this effect remain unclear. The current hypotheses regarding the *NOD2*-related regulation of the expression of mucins and defensins involve the activation of the NF- $\kappa$ B transcription factor and expression of trypsins. Also, *NOD2* may somehow be involved in the activation of the transcription factor signal transducer and activator of transcription 3 (STAT3) by interacting directly with NADH:ubiquinone oxidoreductase subunit A13 (NDUFA13), the expression of which was significantly reduced in the mucosa of patients with Crohn's disease.

Intelectin 1 (ITLN1) expression levels in serum and cells of the colon are decreased in Crohn's disease patients. ITLN1 is a soluble lectin that recognizes galactofuranose in carbohydrate chains of the bacterial cell wall. ITLN1 in healthy Paneth cell may inhibit NF- $\kappa$ B activation. Single nucleotide polymorphism in *ITLN1* was associated with the incidence of Crohn's disease and asthma. Transcription factor 7 like 2 (TCF7L2) is another gene associated with Crohn's disease and impaired Paneth cell antimicrobial function. Understanding the signaling of WNT-TCF7L2, TLR4, and other proteins in Paneth cells in Crohn's disease requires further study ([Koslowski et al., 2009](#); [Moehle et al., 2006](#); [Scharl and Rogler, 2012](#); [Wehkamp et al., 2005](#)).

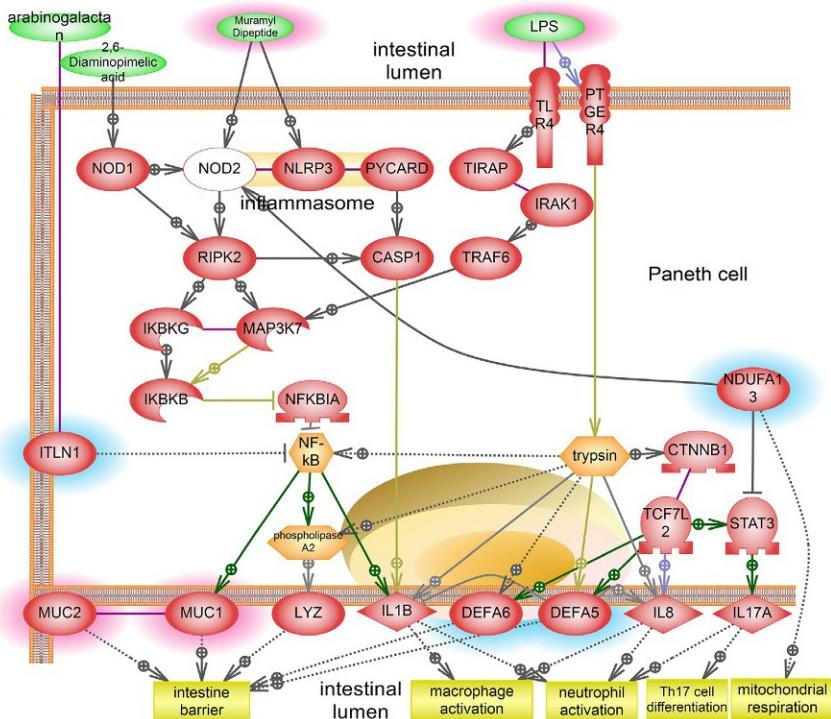


FIG. 2 Pathway 2: Paneth cell dysfunction in Crohn's disease.

## Pathway 3

### Autophagy dysfunction in Crohn's disease (Fig. 3)

#### Incoming signals

Patients with Crohn's disease-associated *NOD2* and autophagy-related 16 like 1 (ATG16L1) mutations have decreased autophagic responses to infection and ineffective elimination of ingested pathogenic bacteria and therefore exhibit free bacterial expansion. In healthy dendritic cell, macrophages and other intestine cells, *NOD2*, and *ATG16L1* are involved in autophagy and consequently in the trafficking of ingested organisms to lysosomes where they are eliminated.

#### Outcome effects

Generally, through autophagy, antigens from pathogens are transmitted to the MHC class II protein complex and in turn activate the T cell- and B cell-mediated immune response. The reduced levels of internalization of bacteria by autophagolysosomes in macrophages and dendritic cells promote the entry of pathogenic organisms or their component parts from the terminal ileum into the lamina propria through Peyer's patches, thereby initiating Crohn's-like inflammation. Although dendritic cells with mutations in the *ATG16L1* gene from patients with Crohn's disease can induce proinflammatory T-cell differentiation, they do not induce regulatory T cells (T(regs)), which would suppress mucosal inflammation.

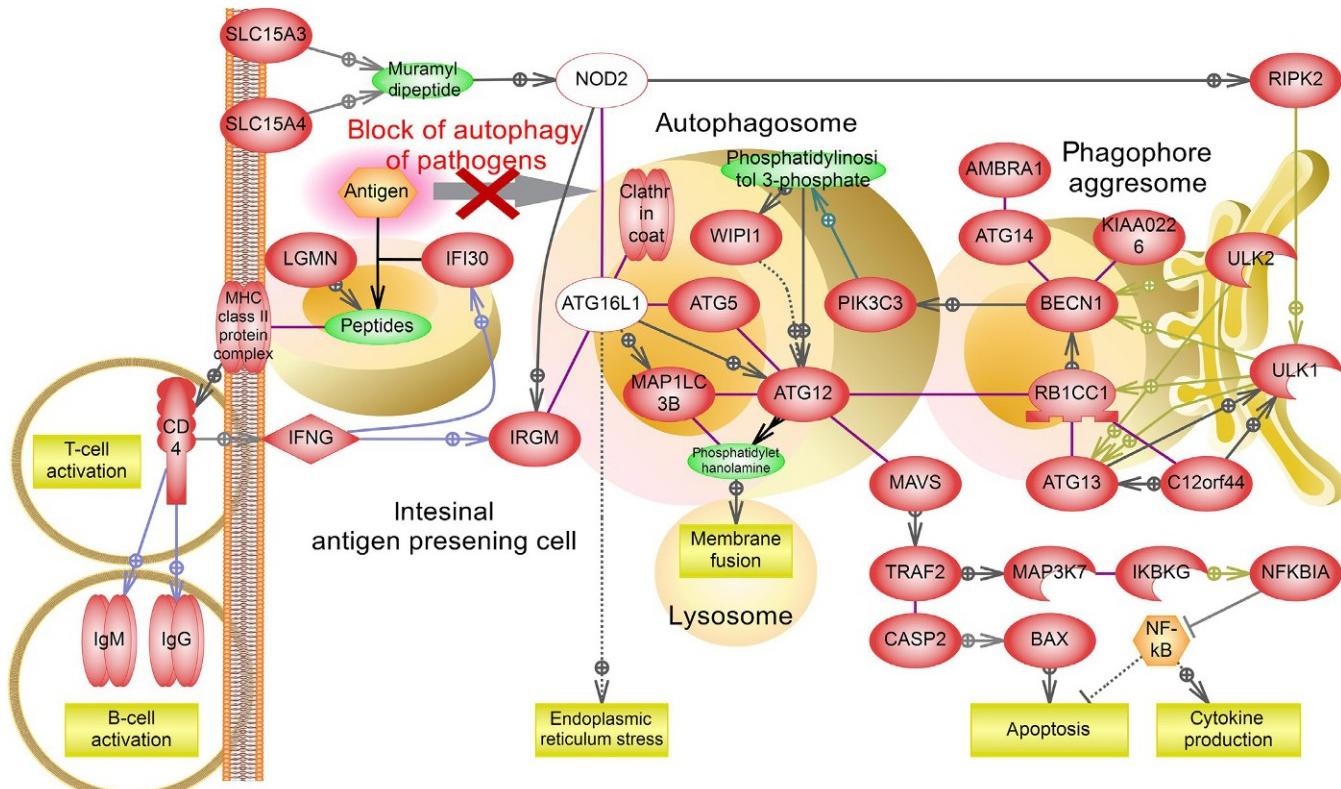
#### Signaling

The activation of *NOD2* by the bacterial muramyl dipeptide induces an autophagy process that requires proteins, receptor-interacting serine/threonine kinase 2 (RIPK2), phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3), autophagy-related 5 (ATG5), and ATG16L1. Two endolysosomal peptide transporters, solute carrier family 15 member 3 (SLC15A3) and solute carrier family 15 member 4 (SLC15A4), mediate the transport of bacterially derived components, such as the muramyl dipeptide (MDP) within the intestinal cells. SLC15A3 and SLC15A4 are expressed at high levels by dendritic cells after the stimulation of TLR and *NOD2* signaling.

Also, both *ATG16L1* and *NOD2* may alter NF- $\kappa$ B activation and the apoptosis of macrophages through TNF receptor-associated factor 2 (TRAF2) signaling.

Finally, patients with Crohn's diseases carrying the *ATG16L1* (T300A, rs2241880) risk variant frequently exhibit endoplasmic reticulum stress (ER stress) and an unfolded protein response (UPR) in Paneth cells (Adolph et al., 2013; Caprilli and Frieri, 2009; Casanova and Abel, 2009; Chu et al., 2016; Cooney et al., 2010; Deuring et al., 2014; Nakamura et al., 2014; Smith et al., 2009).

## II. Human disease pathways



**FIG. 3** Pathway 3: Autophagy dysfunction in Crohn's disease.

## References

- Disease number #266600, #605956, and others Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code K50. Diseases of the digestive system (K00-K93). (ICD-10, <https://icdlist.com>). ICD-11: disease code DD70.
- Adolph, T.E., Tomczak, M.F., Niederreiter, L., Ko, H.-J., Böck, J., Martinez-Naves, E., Glickman, J.N., Tschurtschenthaler, M., Hartwig, J., Hosomi, S., Flak, M.B., Cusick, J.L., Kohno, K., Iwawaki, T., Billmann-Born, S., Raine, T., Bharti, R., Lucius, R., Kweon, M.-N., Marciniaik, S.J., Choi, A., Hagen, S.J., Schreiber, S., Rosenstiel, P., Kaser, A., Blumberg, R.S., 2013. Paneth cells as a site of origin for intestinal inflammation. *Nature* 503, 272–276. <https://doi.org/10.1038/nature12599>.
- Barnich, N., Carvalho, F.A., Glasser, A.-L., Darcha, C., Jantscheff, P., Allez, M., Peeters, H., Bommelaer, G., Desreumaux, P., Colombel, J.-F., Darfeuille-Michaud, A., 2007. CEACAM6 acts as a receptor for adherent-invasive *E. coli*, supporting ileal mucosa colonization in Crohn disease. *J. Clin. Invest.* 117, 1566–1574. <https://doi.org/10.1172/JCI30504>.
- Brain, O., Owens, B.M.J., Pichulik, T., Allan, P., Khatamzas, E., Leslie, A., Steevels, T., Sharma, S., Mayer, A., Catuneanu, A.M., Morton, V., Sun, M.-Y., Jewell, D., Coccia, M., Harrison, O., Maloy, K., Schönenfeldt, S., Bornschein, S., Liston, A., Simmons, A., 2013. The intracellular sensor NOD2 induces microRNA-29 expression in human dendritic cells to limit IL-23 release. *Immunity* 39, 521–536. <https://doi.org/10.1016/j.jimmuni.2013.08.035>.
- Cantó, E., Moga, E., Ricart, E., Garcia-Bosch, O., Garcia-Planella, E., Juarez, C., Vidal, S., 2009. MDP-induced selective tolerance to TLR4 ligands: impairment in NOD2 mutant Crohn's disease patients. *Inflamm. Bowel Dis.* 15, 1686–1696. <https://doi.org/10.1002/ibd.21013>.
- Caprilli, R., Frieri, G., 2009. The dyspeptic macrophage 30 years later: an update in the pathogenesis of Crohn's disease. *Dig. Liver Dis. Off. J. Ital. Soc. Gastroenterol. Ital. Assoc. Study Liver* 41, 166–168. <https://doi.org/10.1016/j.dld.2008.09.012>.
- Casanova, J.-L., Abel, L., 2009. Revisiting Crohn's disease as a primary immunodeficiency of macrophages. *J. Exp. Med.* 206, 1839–1843. <https://doi.org/10.1084/jem.20091683>.
- Cavanaugh, J., IBD International Genetics Consortium, 2001. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set: Crohn disease and chromosome 16. *Am. J. Hum. Genet.* 68, 1165–1171. <https://doi.org/10.1086/320119>.
- Chu, H., Khosravi, A., Kusumawardhani, I.P., Kwon, A.H.K., Vasconcelos, A.C., Cunha, L.D., Mayer, A.E., Shen, Y., Wu, W.-L., Kambal, A., Targan, S.R., Xavier, R.J., Ernst, P.B., Green, D.R., McGovern, D.P.B., Virgin, H.W., Mazmanian, S.K., 2016. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science* 352, 1116–1120. <https://doi.org/10.1126/science.aad9948>.
- Cooney, R., Baker, J., Brain, O., Danis, B., Pichulik, T., Allan, P., Ferguson, D.J.P., Campbell, B.J., Jewell, D., Simmons, A., 2010. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. *Nat. Med.* 16, 90–97. <https://doi.org/10.1038/nm.2069>.
- Deuring, J.J., Fuhrer, G.M., Konstantinov, S.R., Peppelenbosch, M.P., Kuipers, E.J., de Haar, C., van der Woude, C.J., 2014. Genomic ATG16L1 risk allele-restricted Paneth cell ER stress in quiescent Crohn's disease. *Gut* 63, 1081–1091. <https://doi.org/10.1136/gutjnl-2012-303527>.
- Ferri, F.F., 2017. Ferri's Clinical Advisor 2017. 5 Books in 1.
- Festen, E.A.M., Weersma, R.K., 2014. How will insights from genetics translate to clinical practice in inflammatory bowel disease? *Best Pract. Res. Clin. Gastroenterol.* 28, 387–397. <https://doi.org/10.1016/j.bpg.2014.04.002>.
- Franchimont, N., Reenaers, C., Lambert, C., Belaiche, J., Bours, V., Malaise, M., Delvenne, P., Louis, E., 2004. Increased expression of receptor activator of NF- $\kappa$ B ligand (RANKL), its receptor RANK and its decoy receptor osteoprotegerin in the

- colon of Crohn's disease patients. *Clin. Exp. Immunol.* 138, 491–498. <https://doi.org/10.1111/j.1365-2249.2004.02643.x>.
- Koslowski, M.J., Kübler, I., Chamaillard, M., Schaeffeler, E., Reinisch, W., Wang, G., Beisner, J., Teml, A., Peyrin-Biroulet, L., Winter, S., Herrlinger, K.R., Rutgeerts, P., Vermeire, S., Cooney, R., Fellermann, K., Jewell, D., Bevins, C.L., Schwab, M., Stange, E.F., Wehkamp, J., 2009. Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) putative promoter region are associated with small intestinal Crohn's disease. *PLoS One* 4, e4496. <https://doi.org/10.1371/journal.pone.0004496>.
- Kramer, M., Netea, M.G., de Jong, D.J., Kullberg, B.J., Adema, G.J., 2006. Impaired dendritic cell function in Crohn's disease patients with NOD2 3020insC mutation. *J. Leukoc. Biol.* 79, 860–866. <https://doi.org/10.1189/jlb.0805484>.
- Lovato, P., Brender, C., Agnholt, J., Kelsen, J., Kaltoft, K., Svejgaard, A., Eriksen, K.W., Woetmann, A., Ødum, N., 2003. Constitutive STAT3 activation in intestinal T cells from patients with Crohn's disease. *J. Biol. Chem.* 278, 16777–16781. <https://doi.org/10.1074/jbc.M207999200>.
- Marks, D.J.B., Rahman, F.Z., Sewell, G.W., Segal, A.W., 2010. Crohn's disease: an immune deficiency state. *Clin. Rev. Allergy Immunol.* 38, 20–31. <https://doi.org/10.1007/s12016-009-8133-2>.
- Moehle, C., Ackermann, N., Langmann, T., Aslanidis, C., Kel, A., Kel-Margoulis, O., Schmitz-Madry, A., Zahn, A., Stremmel, W., Schmitz, G., 2006. Aberrant intestinal expression and allelic variants of mucin genes associated with inflammatory bowel disease. *J. Mol. Med.* 84, 1055–1066. <https://doi.org/10.1007/s00109-006-0100-2>.
- Nakamura, N., Lill, J.R., Phung, Q., Jiang, Z., Bakalarski, C., de Mazière, A., Klumperman, J., Schlatter, M., Delamarre, L., Mellman, I., 2014. Endosomes are specialized platforms for bacterial sensing and NOD2 signalling. *Nature* 509, 240–244. <https://doi.org/10.1038/nature13133>.
- Niess, J.H., 2008. Role of mucosal dendritic cells in inflammatory bowel disease. *World J. Gastroenterol.* 14, 5138–5148.
- Pizarro, T.T., Michie, M.H., Bentz, M., Woraratanaadharm, J., Smith, M.F., Foley, E., Moskaluk, C.A., Bickston, S.J., Cominelli, F., 1999. IL-18, a novel immunoregulatory cytokine, is up-regulated in Crohn's disease: expression and localization in intestinal mucosal cells. *J. Immunol.* 162, 6829–6835.
- Scharl, M., Rogler, G., 2012. Inflammatory bowel disease: dysfunction of autophagy? *Dig. Dis.* 30 (Suppl. 3), 12–19. <https://doi.org/10.1159/000342588>.
- Smith, A.M., Rahman, F.Z., Hayee, B., Graham, S.J., Marks, D.J.B., Sewell, G.W., Palmer, C.D., Wilde, J., Foxwell, B.M.J., Gloger, I.S., Sweeting, T., Marsh, M., Walker, A.P., Bloom, S.L., Segal, A.W., 2009. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J. Exp. Med.* 206, 1883–1897. <https://doi.org/10.1084/jem.20091233>.
- Strober, W., Kitani, A., Fuss, I., Asano, N., Watanabe, T., 2008. The molecular basis of NOD2 susceptibility mutations in Crohn's disease. *Mucosal Immunol.* 1 (Suppl. 1), S5–S9. <https://doi.org/10.1038/mi.2008.42>.
- Tozer, P.J., Whelan, K., Phillips, R.K.S., Hart, A.L., 2009. Etiology of perianal Crohn's disease: role of genetic, microbiological, and immunological factors. *Inflamm. Bowel Dis.* 15, 1591–1598. <https://doi.org/10.1002/ibd.21026>.
- van Heel, D.A., Fisher, S.A., Kirby, A., Daly, M.J., Rioux, J.D., Lewis, C.M., Genome Scan Meta-Analysis Group of the IBD International Genetics Consortium, 2004. Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. *Hum. Mol. Genet.* 13, 763–770. <https://doi.org/10.1093/hmg/ddh090>.
- Wehkamp, J., Schmid, M., Fellermann, K., Stange, E.F., 2005. Defensin deficiency, intestinal microbes, and the clinical phenotypes of Crohn's disease. *J. Leukoc. Biol.* 77, 460–465. <https://doi.org/10.1189/jlb.0904543>.
- Yamazaki, K., Takazoe, M., Tanaka, T., Kazumori, T., Nakamura, Y., 2002. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. *J. Hum. Genet.* 47, 469–472. <https://doi.org/10.1007/s100380200067>.

## CHAPTER

## 10.2

## Ulcerative colitis

*Ulcerative colitis is a chronic disorder that affects the digestive system. This condition is characterized by abnormal inflammation of the inner surface of the rectum and colon, which make up most of the length of the large intestine. The inflammation usually causes open sores (ulcers) to develop in the large intestine. (Genetics Home Reference, <https://ghr.nlm.nih.gov>).*

Ulcerative colitis (UC) is an idiopathic, remitting and relapsing, chronic inflammatory bowel disease that starts in the rectum and extends proximally. Accumulating evidence suggests that it may result from an inappropriate inflammatory response to environmental triggers and immune dysregulation involving CD4+ T-cell Th2 response in a genetically susceptible host. (*Ferri, 2017*, p. 1).

A breakdown of the intestinal barrier that protects the body from host microbiota and toxins allows bacteria and toxic molecules penetrate the tissues and trigger an immune reaction. In patient with ulcerative colitis, this immune response leads to chronic uncontrolled inflammation of the inner surface of the rectum and colon and the digestive problems. A variety of genetic and environmental factors are likely involved in the development of ulcerative colitis. Recent studies have identified variations in dozens of genes that may be linked to the protective function of the intestines and ulcerative colitis; however, the role of these variations is not completely understood (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Ulcerative colitis initiates with the disruption of the epithelial barrier of the rectum and colon resulting in increased uptake of luminal microbiota and antigens. Dysfunction of intestine epithelial cells reduces the defense abilities of the mucosal layer and causes a loss of mucosal tolerance to luminal pathogens.

**Pathway 1.** *Intestine epithelial cell dysfunction in ulcerative colitis (Fig. 4).* Intestine epithelial cells and antigen-presenting cells of the intestine, such as dendritic cells and macrophages, recognize commensal microbiota through toll-like receptors (TLRs) and Nod-like (NOD) receptors, become activated, and in turn secrete cytokines. Cytokines stimulate

T-helper 2 cells (Th2) differentiation and their proinflammatory response. The atypically strong Th2 cell response in patients with ulcerative colitis is cytotoxic and results in necrosis of the inner intestine wall.

**Pathway 2.** *Intestine epithelial cells drive immunological responses in ulcerative colitis* ([Fig. 5](#)).

Inflamed mucosa amplifies the extravasation of leukocytes and their accumulation in the intestinal mucosa. This process is controlled by a family of cell adhesion molecules (CAMs) including the intercellular adhesion molecule 1 (ICAM1), platelet and endothelial cell adhesion molecule 1 (PECAM1), mucosal vascular addressin cell adhesion molecule 1 (MADCAM1), the selectins, and the integrins.

**Pathway 3.** *Leukocyte migration toward the endothelial cells in the intestine microvasculature* ([Fig. 6](#)).

Studies have described more than 100 variations associated with ulcerative colitis. However, much more additional work is needed to confirm the associations and the disease-specific manifestations for many of declared variations. Sometimes, it is impossible to separate ulcerative colitis from Crohn's disease and other manifestations of inflammatory bowel diseases in data analysis and in diagnosis ([Anderson et al., 2011](#); [Kang et al., 2016](#); [Thompson and Lees, 2011](#)). So, inflammatory bowel diseases share many reported associated variations.

With each new genome-wide association study (GWAS), the list of mutated genes associated with the ulcerative colitis has changed. Obvious challenges exist when the scientific community tries to identify the variations most significantly associated with ulcerative colitis in different human populations ([Jung and Hugot, 2009](#)). This unsteady state of knowledge indicates that we are at the very beginning of understanding the mechanisms of this disease.

**Pathway 4.** *Polymorphisms associated with inflammatory bowel diseases* ([Fig. 7](#)).

Genes with mutations currently associated with ulcerative colitis filtered by reference count ([Fig. 8](#)).

## Key cellular contributors and processes

Interleukins

Protein or gene

Interleukins are a subgroup of a large group of extracellular signaling molecules called cytokines. Interleukins are low-molecular-weight proteins involved in the functioning of both the adaptive and innate immune system.

Paneth cell

Cell

Paneth cells are specialized secretory epithelial cells of the small intestine that produce antimicrobial peptides and are key players in the intestinal innate immune defense.

Proinflammatory cytokines

Protein or gene

Cytokines are a broad list of small proteins released by immune cells, which participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony stimulating factor (GMCS-F), and others), secreted primarily by macrophages and T-helper cells, upregulate proinflammatory reactions.

Toll-like receptors

Protein or gene

Toll-like receptors belong to a family of membrane proteins that can directly bind microbial molecules or proteins and initiate the innate immune response.

Type 1 and Type 2 T-helper cells

Cell

Type 1 and Type 2 T-helper cell (Th1, Th2 cell) are cells of the T-cell lineage that protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) via stimulation of B-cell maturation and activation of other immune cells.

## Pathway 1

### Intestine epithelial cell dysfunction in ulcerative colitis ([Fig. 4](#))

#### Incoming signals

The mucosal layer, or the defensive part of the *gastrointestinal wall*, consists of the lamina propria, epithelium, and a muscle layer.

Intestinal epithelial cells in the mucosal layer stand in the way of penetration by bacteria, viruses, toxins, and antigens through the basement membrane into the lamina propria where immune cells are located. It is reasonable that the intestines must immunologically react to external antigens, but if it responds to each of the alien agents, we would have extensive inflammation. So the right level of sensitivity to pathogens is the key to the health of the gastrointestinal tract.

In ulcerative colitis (as well as in a Crohn's disease), presumably, the normal balance between the colon and the commensal flora is not observed.

Intestinal epithelial cells (IECs) include enterocytes, goblet cells, and Paneth cells. IECs have different functions in the context of defense against pathogens, including the secretion of mucus, the secretion of antimicrobial molecules, and immune-regulatory molecules such as cytokines or chemokines. Alterations in the genes implicated in the normal function of the intestinal epithelial cells may be the reason for mucosal barrier disruption and inability to clear pathogenic molecules or organisms in ulcerative colitis. However, there is no solid evidence regarding the exact role and the number of mutated genes in the disease development (see [Pathway 4](#)).

#### Outcome effects

The imbalance between proinflammatory signaling and the secretion of mucus and antibacterial molecules leads to a loss of mucosal tolerance to luminal microbial organisms, and therefore, it initiates the inflammation characteristic of ulcerative colitis.

More than 1000 species of bacteria colonize the human intestine. Obligate anaerobic Firmicutes (Gram-positive) and Bacteroidetes (Gram-negative) organisms account for most intestinal bacteria. Patients with bowel inflammatory diseases have fewer bacteria with antiinflammatory properties (phylum Firmicutes) and more sulfate-reducing bacteria with proinflammatory properties, which can result in the production of toxic hydrogen sulfide ([Zhang et al., 2017](#)).

## Signaling

### **Peroxisome proliferator-activated receptor gamma**

Reduced levels of peroxisome proliferator-activated receptor gamma (PPARG) expression were found in epithelial cells of patients with ulcerative colitis (Annese et al., 2012). The exact mechanism of PPARG action in the epithelial cell response to the infiltrating flora is not known. PPARG could be involved in regulating expression of short and long isoforms of the immune-regulatory cytokine thymic stromal lymphopoietin (TSLP) secreted by IECs in the colonic mucosa of UC patients.

Anthony Martin Mena and his coauthors observed a downregulation of the short isoform TSLP mRNA levels. Other researchers found that inflammation typically upregulated levels of the long isoform TSLP. The shifting balance of short isoform TSLP/long isoform TSLP ratio induces a proinflammatory response in gut disorders. High levels of the short isoform of TSLP may be involved with uncontrolled Th1 type inflammation (by a reduction of interferon gamma (IFNG) production), such as in patients with Crohn's disease. Upregulated levels of the long isoform of TSLP in patients with ulcerative colitis may trigger Th2 type inflammation (Fornasa et al., 2015).

Also, there is a hypothesis that downregulation of PPARG may shift the balance leading to overactivation of toll-like receptor 4 (TLR4) signaling, resulting in the loss of mucosal tolerance to bacterially derived molecules and in the increased expression of the proinflammatory tumor necrosis factor (TNF) and interleukin 33 (IL-33) (TLR-dependent signaling is described in the Pathway 2) (Martin Mena et al., 2017; Zou et al., 2009).

### **Cell-to-cell junctions**

Epithelium dysfunction in ulcerative colitis may also be triggered by the breakdown of junctions between IECs and the dysregulation of apical and basolateral intestinal epithelial cell polarity. Details of the regulation of IEC cytoskeleton are under investigation.

However, there are some known facts. For example, cadherin 1 (CDH1), which is a cell adhesion protein with crucial roles in cell-cell contacts, has low expression levels in patients with Crohn's disease and ulcerative colitis (Schneider et al., 2010). Polymorphisms in the genes encoding actin-related protein 2/3 complex subunit 2 (ARPC2), extracellular matrix protein 1 (ECM1), anterior gradient homolog 2 (AGR2), and other proteins involved in the regulation of epithelium architecture were associated with an increased risk of ulcerative colitis (Rosenstiel et al., 2009).

### **Short-chain fatty acids**

The production of short-chain fatty acids (SCFAs) was decreased in patients with inflammatory bowel diseases because of decreased levels of the bacterium *Faecalibacterium prausnitzii* or downregulated expression of solute carrier family 16 member 1 (SLC16A1) in the gut (Goto and Ivanov, 2013; Thibault et al., 2007).

SCFAs, like butyrate, are an energy source for the colonic epithelium and can deliver antiinflammatory effects by inhibiting histone deacetylases (not shown) and activating free fatty acid receptor 2 (FFAR2) or free fatty acid receptor 3 (FFAR3) signaling. Butyrate/hydroxycarboxylic acid receptor 2 (HCAR2) signaling has tumor suppressor effects in colon cancer (Goto and Ivanov, 2013). SCFAs can hypothetically inhibit inflammation by regulating the secretion of cytokines and mucin.

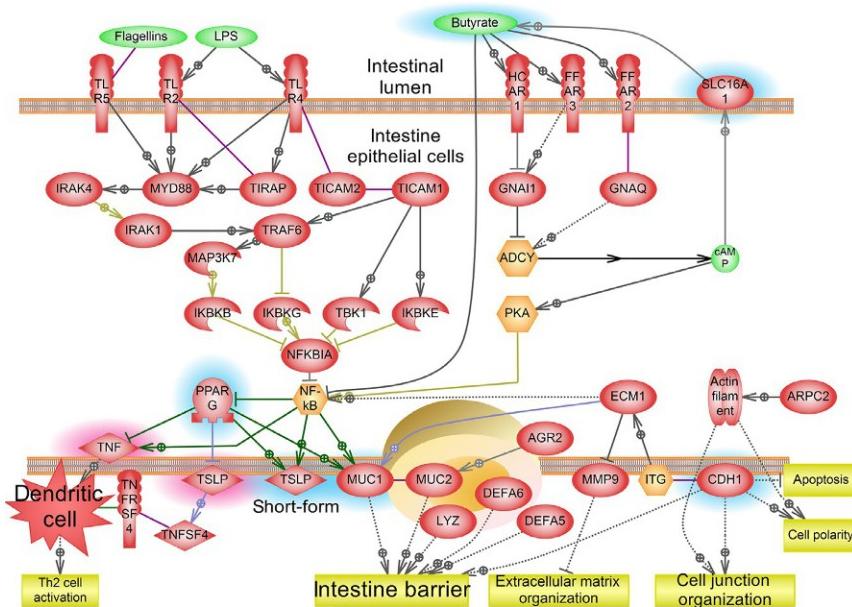


FIG. 4 Pathway 1: Intestine epithelial cell dysfunction in ulcerative colitis.

## Pathway 2

### Intestine epithelial cells drive immunological responses in ulcerative colitis (Fig. 5)

#### Incoming signals

The epithelial barrier, covered by a mucinous layer, is the first line of defense in the mucosal immune system of the human gut. Processes are described in [Pathway 1](#), such as decreased production of mucin 2 (MUC2) in ulcerative colitis, resulting in damage to the epithelial barrier. This barrier loss enables increased uptake of luminal antigens and results in increased permeability.

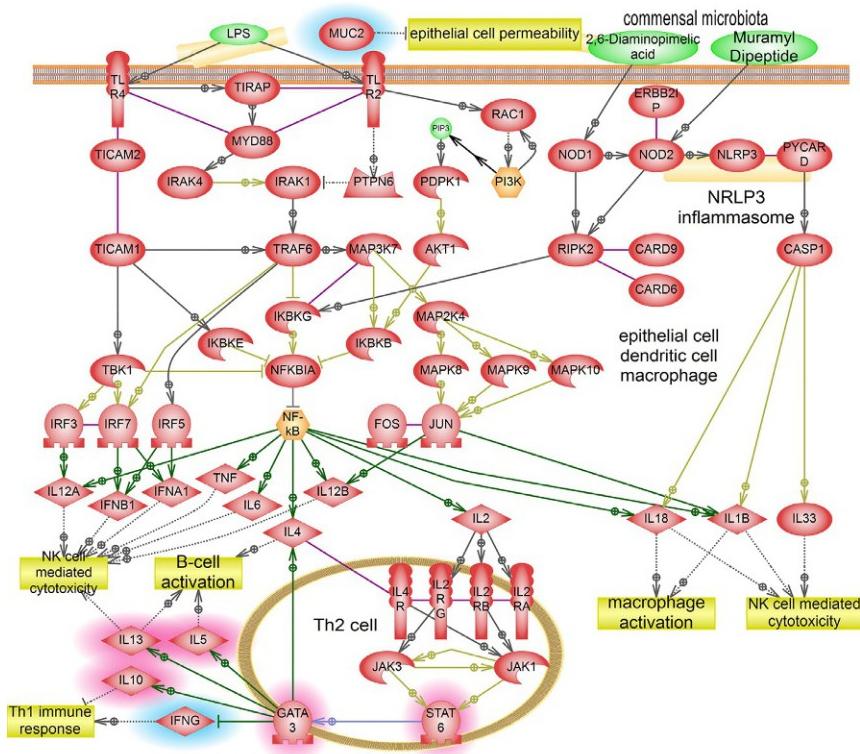
#### Outcome effects

Intestinal Th2 cells react more intensely in patients with ulcerative colitis than their Th1 immune response. Th2 cells demonstrate overexpression of signal transducer and activator of transcription 6 (STAT6) and GATA binding protein 3 (GATA3), major transcription factors responsible for the T-cell function, and they are characterized by the increased production of interleukins 4, 5, 13, and 10 (IL-4, IL-5, IL-13, and IL-10) and decreased production of interferon gamma (IFNG). The atypical T-helper 2 cell response in patients with ulcerative colitis has cytotoxic effects against epithelial cells by activating NK cell-mediated cytotoxicity. NK cells damage intestinal tissue directly leading to necrosis.

#### Signaling

Weakening of the epithelial barrier leads to the amplified uptake of luminal antigens. IECs, dendritic cells, and macrophages of the mucosal layer recognize commensal microbiota through molecular pattern recognition receptors (PRRs), including the toll-like receptors and NOD-like receptors. Toll-like receptor 4 (TLR4) and toll-like receptor 2 (TLR2) expression levels can be noticeably increased in the lamina propria cells of patients with ulcerative colitis. The association of polymorphisms in the genes encoding proteins involved with the TLR4/TLR2 and nucleotide-binding oligomerization domain containing 2 (NOD2) cascades with ulcerative colitis has been reported, but additional confirmation is required. Activation of toll-like receptors and NOD-like receptors triggers activation of the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B), the transcription factor activator protein 1 (JUN/FOS), and other transcription factors that are important in the stimulation of both innate and adaptive immune responses. For example, NF- $\kappa$ B stimulates the transcription of proinflammatory cytokines

such as tumor necrosis factor (TNF); the interleukins 12, 23, and 6 (IL-12, IL-23, and IL-6); and interleukin 1B (IL-1B). The NOD2 related inflammasome cascade activates expression of the IL-33, IL-18, and IL-1B cytokines. Secreted proteins stimulate T-helper 2 cell differentiation and their proinflammatory response. Also, dendritic cells and macrophages can process and present antigens to naive CD4+ T cells and initiate the differentiation of naive CD4+ T cells into Th2 effector cells (for inflammasome cascade and antigen-presenting description, see Crohn's disease pathways) ([Bamias et al., 2011](#); [Cho, 2008](#); [Rodriguez-Bores et al., 2007](#); [Sarlos et al., 2014](#); [Strober and Fuss, 2011](#); [Verma et al., 2013](#)).



**FIG. 5** Pathway 2: Intestine epithelial cells drive immunological responses in ulcerative colitis.

## Pathway 3

### Leukocyte migration toward the endothelial cells in the intestine microvasculature ([Fig. 6](#))

#### Incoming signals

Inflamed mucosa releases molecules that attract circulating leucocytes from the systemic circulation to the site of inflammation.

Proinflammatory cytokines from dysfunctional intestinal endothelial cells upregulate the expression of a family of cell adhesion molecules on mucosal blood vessels. Those adhesion molecules, in turn, promote leucocyte adhesion and accumulation in the inflamed intestinal mucosa.

#### Outcome effects

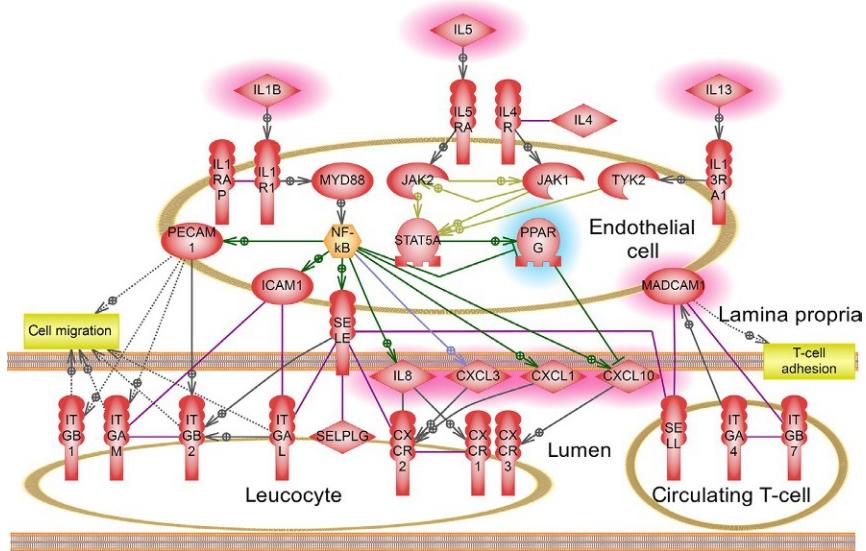
Recruitment of circulating leucocytes results in an increase of the inflammatory response in patients with ulcerative colitis.

#### Signaling

Cell adhesion molecules mediate the extravasation of leukocytes and their accumulation in the inflamed intestinal mucosa. This process is controlled by a family of CAM molecules including ICAM1, PECAM1 and MADCAM1, the selectins, and the integrins. ICAM1 and PECAM1 are cell surface glycoproteins found on vascular endothelial cells that together facilitate a transendothelial migration of circulating leukocytes through the lamina propria. The protein SELE (E-selectin) is expressed on endothelial cells in response to proinflammatory cytokines. It supports the rolling of leukocytes at sites of inflammation and tissue injury. SELL (L-selectin) is expressed on regular naive T and B cells, leukocytes, and natural killer cells, and it is normally involved in the adhesion of T cells to endothelial cells.

Expression of the MADCAM1 protein is enhanced in inflamed intestine. Circulating gut-specific T cells bearing the integrins alpha-4 and beta-7 (ITGA4, ITGB7) bind to MADCAM1 on the microvasculature endothelial cells. MADCAM1 also interacts with SELL (L-selectin) integrins, to mediate lymphocyte homing.

Inflamed mucosa releases such chemoattractants as C-X-C motif chemokine ligand 8 (CXCL8), C-X-C motif chemokine ligand 1 CXCL1, and C-X-C motif chemokine ligand 3 (CXCL3) that together can upregulate the expression of a family of CAM on the vascular endothelium of mucosal blood vessels ([Ordás et al., 2012; Sarlos et al., 2014](#)).



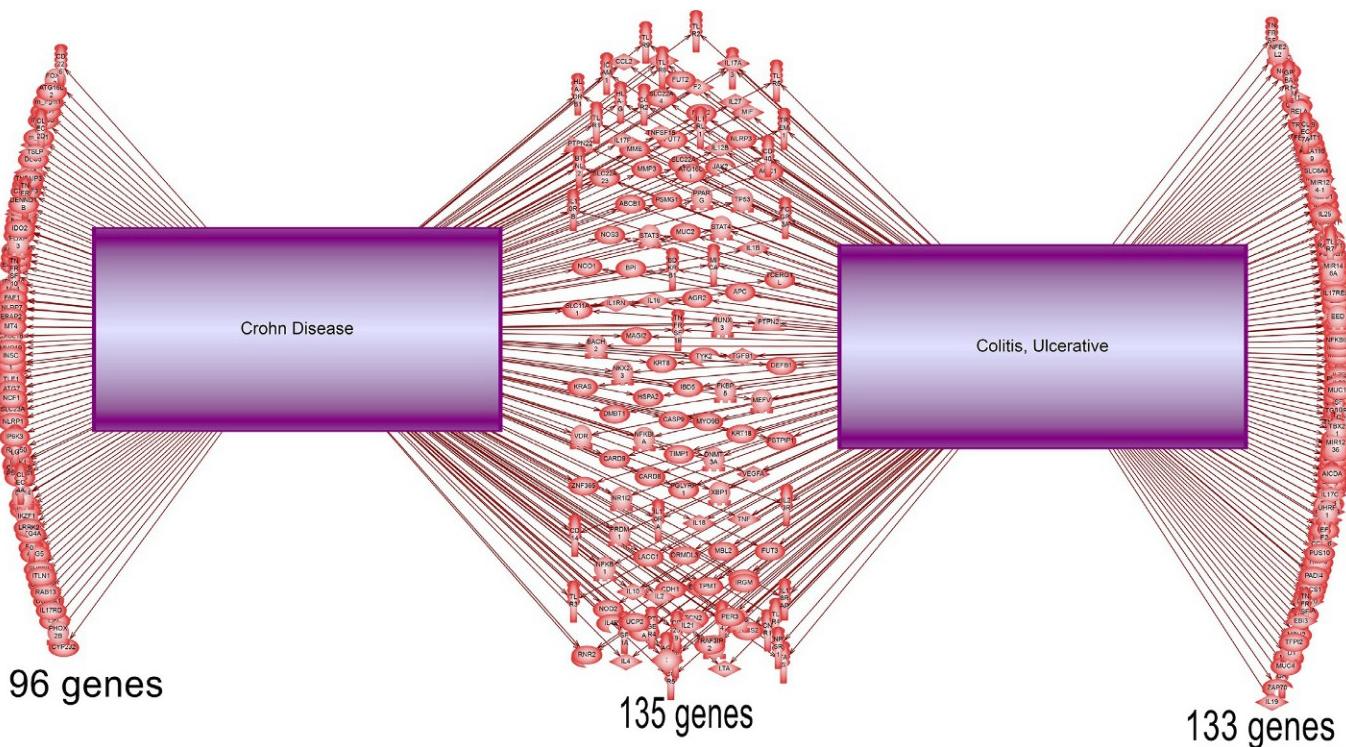
**FIG. 6** Pathway 3: Leukocyte migration toward the endothelial cells in the intestine microvasculature.

## Pathway 4

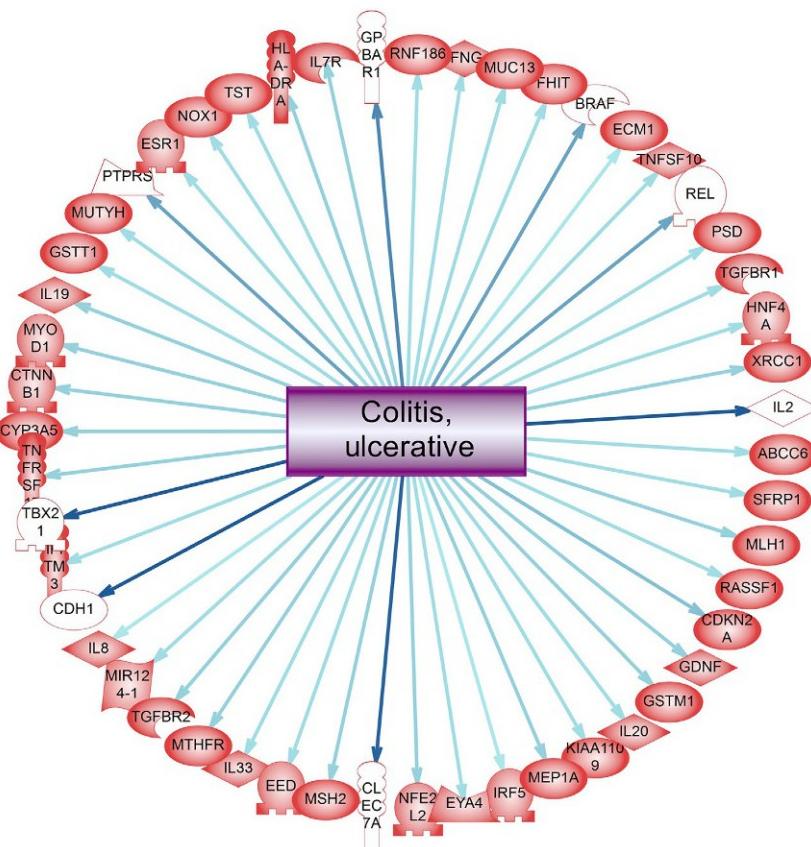
### Polymorphisms associated with inflammatory bowel diseases ([Fig. 7](#))

The genes encoding T-box 2 (TBX2), protein tyrosine phosphatase, receptor type S (PTPRS), G protein–coupled bile acid receptor 1 (GPBAR1), B-Raf proto-oncogene, serine/threonine kinase (BRAF), REL proto-oncogene (REL, NF- $\kappa$ B subunit), interleukin 2 (IL-2), C-type lectin domain containing 7A (CLEC7A), and cadherin 1 (CDH1) are mentioned in more than five publications regarding the association of mutations with ulcerative colitis.

[Fig. 8](#) represents genes with mutations currently associated with ulcerative colitis filtered by reference count. When the arrow's color in [Fig. 8](#) is darker, there is more confidence in the association between the indicated gene and the disease.



**FIG. 7** Pathway 4: Polymorphisms associated with inflammatory bowel diseases.



**FIG. 8** Genes with mutations currently associated with ulcerative colitis.

## References

- Disease number #601458 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code K51. Diseases of the digestive system (K00-K93). (ICD-10, <https://icdlist.com>). ICD-11: disease code DD71.
- Anderson, C.A., Boucher, G., Lees, C.W., Franke, A., D'Amato, M., Taylor, K.D., Lee, J.C., Goyette, P., Imielinski, M., Latiiano, A., Lagacé, C., Scott, R., Amininejad, L., Bumpstead, S., Baidoo, L., Baldassano, R.N., Barclay, M., Bayless, T.M., Brand, S., Büning, C., Colombel, J.-F., Denson, L.A., De Vos, M., Dubinsky, M., Edwards, C., Ellinghaus, D., Fehrmann, R.S.N., Floyd, J.A.B., Florin, T., Franchimont, D., Franke, L., Georges, M., Glas, J., Glazer, N.L., Guthery, S.L., Harritunians, T., Hayward, N.K., Hugot, J.-P., Jobin, G., Laukens, D., Lawrence, I., Lémann, M., Levine, A., Libioulle, C., Louis, E., McGovern, D.P., Milla, M., Montgomery, G.W., Morley, K.I., Mowat, C., Ng, A., Newman, W., Ophoff, R.A., Papi, L., Palmieri, O., Peyrin-Biroulet, L., Panés, J., Phillips, A., Prescott, N.J., Proctor, D.D., Roberts, R., Russell, R., Rutgeerts, P., Sanderson, J., Sans, M., Schumm, P., Seibold, F., Sharma, Y., Simms, L.A., Seielstad, M., Steinhart, A.H., Targan, S.R., van den Berg, L.H., Vatn, M., Verspaget, H., Walters, T., Wijmenga, C., Wilson, D.C., Westra, H.-J., Xavier, R.J., Zhao, Z.Z., Ponsioen, C.Y., Andersen, V., Torkvist, L., Gazouli, M., Anagnou, N.P., Karlsen, T.H., Kupcinskas, L., Sventoraityte, J., Mansfield, J.C., Kugathasan, S., Silverberg, M.S., Halfvarson, J., Rotter, J.I., Mathew, C.G., Griffiths, A.M., Gearry, R., Ahmad, T., Brant, S.R., Chamaillard, M., Satsangi, J., Cho, J.H., Schreiber, S., Daly, M.J., Barrett, J.C., Parkes, M., Annese, V., Hakonarson, H., Radford-Smith, G., Duerr, R.H., Vermeire, S., Weersma, R.K., Rioux, J.D., 2011. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat. Genet.* 43, 246–252. <https://doi.org/10.1038/ng.764>.
- Annese, V., Rogai, F., Settesoldi, A., Bagnoli, S., 2012. PPAR $\gamma$  in inflammatory bowel disease. *PPAR Res.* 2012, 620839. <https://doi.org/10.1155/2012/620839>.
- Bamias, G., Kaltsas, G., Ladas, S.D., 2011. Cytokines in the pathogenesis of ulcerative colitis. *Discov. Med.* 11, 459–467.
- Cho, J.H., 2008. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat. Rev. Immunol.* 8, 458–466. <https://doi.org/10.1038/nri2340>.
- Ferri, F.F., 2017. Ferri's Clinical Advisor 2017. 5 Books in 1.
- Fornasa, G., Tsilingiri, K., Caprioli, F., Botti, F., Mapelli, M., Meller, S., Kislat, A., Homey, B., Di Sabatino, A., Sonzogni, A., Viale, G., Diaferia, G., Gori, A., Longhi, R., Penna, G., Rescigno, M., 2015. Dichotomy of short and long thymic stromal lymphopoietin isoforms in inflammatory disorders of the bowel and skin. *J. Allergy Clin. Immunol.* 136, 413–422. <https://doi.org/10.1016/j.jaci.2015.04.011>.
- Goto, Y., Ivanov, I.I., 2013. Intestinal epithelial cells as mediators of the commensal-host immune crosstalk. *Immunol. Cell Biol.* 91, 204–214. <https://doi.org/10.1038/icb.2012.80>.
- Jung, C., Hugot, J.-P., 2009. Inflammatory bowel diseases: the genetic revolution. *Gastroenterol. Clin. Biol.* 33 (Suppl. 3), S123–S130. [https://doi.org/10.1016/S0399-8320\(09\)73147-1](https://doi.org/10.1016/S0399-8320(09)73147-1).
- Kang, K., Bae, J.-H., Han, K., Kim, E.S., Kim, T.-O., Yi, J.M., 2016. A genome-wide methylation approach identifies a new hypermethylated gene panel in ulcerative colitis. *Int. J. Mol. Sci.* 17. <https://doi.org/10.3390/ijms17081291>.
- Martin Mena, A., Langlois, A., Speca, S., Schneider, L., Desreumaux, P., Dubuquoy, L., Bertin, B., 2017. The expression of the short isoform of thymic stromal lymphopoietin in the colon is regulated by the nuclear receptor peroxisome proliferator activated receptor-gamma and is impaired during ulcerative colitis. *Front. Immunol.* 8, 1052. <https://doi.org/10.3389/fimmu.2017.01052>.
- Ordás, I., Eckmann, L., Talamini, M., Baumgart, D.C., Sandborn, W.J., 2012. Ulcerative colitis. *Lancet* 380, 1606–1619. [https://doi.org/10.1016/S0140-6736\(12\)60150-0](https://doi.org/10.1016/S0140-6736(12)60150-0).
- Rodriguez-Bores, L., Fonseca, G.-C., Villeda, M.-A., Yamamoto-Furusho, J.-K., 2007. Novel genetic markers in inflammatory bowel disease. *World J. Gastroenterol.* 13, 5560–5570.

- Rosenstiel, P., Sina, C., Franke, A., Schreiber, S., 2009. Towards a molecular risk map—recent advances on the etiology of inflammatory bowel disease. *Semin. Immunol.* 21, 334–345. <https://doi.org/10.1016/j.smim.2009.10.001>.
- Sarlos, P., Kovacs, E., Magyari, L., Banfai, Z., Szabo, A., Javorhazy, A., Melegi, B., 2014. Genetic update on inflammatory factors in ulcerative colitis: review of the current literature. *World J. Gastrointest. Pathophysiol.* 5, 304–321. <https://doi.org/10.4291/wjgp.v5.i3.304>.
- Schneider, M.R., Dahlhoff, M., Horst, D., Hirschi, B., Trülzsch, K., Müller-Höcker, J., Vogelmann, R., Allgäuer, M., Gerhard, M., Steininger, S., Wolf, E., Kolligs, F.T., 2010. A key role for E-cadherin in intestinal homeostasis and Paneth cell maturation. *PLoS One* 5, e14325. <https://doi.org/10.1371/journal.pone.0014325>.
- Strober, W., Fuss, I.J., 2011. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140, 1756–1767. <https://doi.org/10.1053/j.gastro.2011.02.016>.
- Thibault, R., De Coppet, P., Daly, K., Bourreille, A., Cuff, M., Bonnet, C., Mosnier, J.-F., Galmiche, J.-P., Shirazi-Beechey, S., Segain, J.-P., 2007. Down-regulation of the monocarboxylate transporter 1 is involved in butyrate deficiency during intestinal inflammation. *Gastroenterology* 133, 1916–1927. <https://doi.org/10.1053/j.gastro.2007.08.041>.
- Thompson, A.I., Lees, C.W., 2011. Genetics of ulcerative colitis. *Inflamm. Bowel Dis.* 17, 831–848. <https://doi.org/10.1002/ibd.21375>.
- Verma, R., Verma, N., Paul, J., 2013. Expression of inflammatory genes in the colon of ulcerative colitis patients varies with activity both at the mRNA and protein level. *Eur. Cytokine Netw.* 24, 130–138. <https://doi.org/10.1684/ecn.2013.0343>.
- Zhang, M., Sun, K., Wu, Y., Yang, Y., Tso, P., Wu, Z., 2017. Interactions between intestinal microbiota and host immune response in inflammatory bowel disease. *Front. Immunol.* 8, 942. <https://doi.org/10.3389/fimmu.2017.00942>.
- Zou, B., Qiao, L., Wong, B.C.Y., 2009. Current understanding of the role of PPAR $\gamma$  in gastrointestinal cancers. *PPAR Res.* 2009, 816957. <https://doi.org/10.1155/2009/816957>.

## CHAPTER

## 10.3

## Celiac disease

Celiac disease results from an inappropriate T cell-mediated immune response against ingested gluten in genetically predisposed individuals who carry mutations in either the *HLA-DQ2* or *HLA-DQ8* genes.

Celiac disease is a chronic autoimmune disease, with tissue transglutaminase (tTG) suggested as a major autoantigen. (*Ferri and Ferri, 2018*).

Patients with celiac disease have a sensitivity to gliadin, a protein fraction of gluten found in wheat, rye, and barley. Immune responses to gliadin promote an inflammatory reaction, mainly in the upper small intestine, manifested by chronic infiltration of the lamina propria and the epithelium by inflammatory and immune cells resulting in villous atrophy.

Celiac disease is one of the most common complex diseases of the digestive system, resulting from both environmental (gluten) and genetic factors (particular variations in human leukocyte antigen (HLA) and non-HLA genes). CD4+ gluten-specific T-helper (Th) cells in celiac disease recognize deamidated gluten-derived peptides presented by either HLA-DQ2 or HLA-DQ8 as antigens, and they respond to those peptides by expressing high levels of inflammatory cytokines. These cytokines in turn affect the epithelial cells and activate intraepithelial lymphocytes.

**Pathway 1.** *CD4+ T cell and epithelial cell response in celiac disease* ([Fig. 9](#)). Gluten-derived peptides transported across the intestinal epithelium and high level of interleukin-15 promote cytotoxic effects of intraepithelial lymphocytes such as natural killer cells, neutrophils, and cytotoxic T lymphocytes.

**Pathway 2.** *CD8+ T-cell response and mucosal damage in celiac disease* ([Fig. 10](#)).

## Key cellular contributors and processes

### CD4+ T cell

#### Cell

CD4+ T cells are a subtype of T cells (T lymphocytes) that recognize peptides presented on MHC class II molecules of antigen-presenting cells. CD4+ T cells protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) by stimulating B-cell maturation and activation of other immune cells.

### CD8+ T cell or killer T cell

#### Cell

Cytotoxic T cells (killer T cells) are a subtype of T cells (T lymphocytes), which eliminate infected or damaged cells, and antigens recognized by cytotoxic T cells typically come from processed cytosolic proteins. Most cytotoxic T cells express T-cell receptors (TCRs), which can recognize a specific antigen. Cytosolic antigens are bound to class I MHC molecules, and in order for the TCR to bind to the class I MHC molecule, the former must be accompanied by a glycoprotein called CD8.

### Gluten and gliadin

#### Protein or gene

Gluten is a general name for the mixture of storage proteins found in the endosperm of cereal grains. Gluten exposure activates an immune response in susceptible patients causing certain health conditions, such as celiac disease and nonceliac gluten sensitivity. Gluten proteins are classified into two major groups based on their solubility in aqueous alcohol: prolamins and glutelins. Gliadin is a prolamin present in wheat and other cereal grains of the genus *Triticum*. Gliadin causes bread to rise during baking and is thought to be primarily responsible for the negative effects of gluten.

### Proinflammatory cytokines

#### Protein or gene

Cytokines are a broad list of small proteins released by immune cells, which participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony stimulating factor (GMCS-F), and others), secreted primarily by macrophages and T-helper cells, upregulate proinflammatory reactions.

## Pathway 1

### CD4+ T cell and epithelial cell response in celiac disease (Fig. 9)

#### Incoming signals

The intestinal epithelium plays a central role in the pathogenesis of celiac disease. Gliadin can gain access to the basal surface of the epithelium via both trans- and paracellular routes of absorption and therefore interacts directly with the immune system.

CD4+ gluten-specific T-helper (Th) cells recognize the deamidated gluten-derived peptides presented by the human leukocyte antigen HLA-DQ2 or HLA-DQ8 molecules of antigen-presenting cells (APC). Variants of the *HLA-DQ2*, *HLA-DQ8*, *CTLA4*, *ICOS*, and *CD28* genes that increase susceptibility to celiac disease have been identified.

#### Outcome effects

The response of the intestinal epithelium to gliadin leads to the activation of intestinal intraepithelial cytotoxic T lymphocytes and natural killer cells.

Further the deamidated gluten-derived peptides stimulate APC and Th-cell activation that in response express high levels of T-helper cell-type-(Th)1 cytokine such as interleukin-21 (IL-21) and interferon gamma (IFNG). These cytokines affect intestinal epithelial cells and attract natural killer cells (NK), neutrophils, cytotoxic T cells, and other intraepithelial lymphocytes. Attracted and activated cytotoxic T lymphocytes release perforin/granzymes that damage the intestinal epithelium. This response leads directly to tissue remodeling and flattening of the intestinal mucosa, ultimately leading to villous atrophy (see [Pathway 2](#)).

#### Signaling

Tissue transglutaminase 2 (TGM2) is a calcium-dependent enzyme that mediates specific deamidation of gliadins, creating an epitope that binds efficiently to HLA-DQ2, which can then be recognized by gut-derived T cells.

Gluten peptides can be taken up by cells during transcytosis (or retrotranscytosis) of secretory immunoglobulin IgA through the transferrin receptor (TFRC, CD71).

Gluten peptides can probably also be transported across the intestinal epithelium due to activation of the zonulin (haptoglobin, HP) mechanism

of innate immunity. HP is involved in the maintenance of mucosal integrity and the modulation of this intestinal barrier.

CXCR3 (19-mer gliadin binding to chemokine (C-X-C motif) receptor 3) induces the expression of HP. HP is involved in a protein kinase C-mediated rearrangement of the cytoskeleton, downregulation of the tight junction protein 1 (TJP1) and occludin (OCLN), and disruption of cell-to-cell junctions, thereby increasing epithelial tissue permeability.

Intestinal epithelial cells express high levels of interleukin-15 (IL-15) in response to gliadin. That expression leads to the upregulation of protein MHC class I polypeptide-related sequence A (MICA) and the attraction of cytotoxic cells via the killer cell lectin-like receptor K1 (KLRK1) (Fasano, 2006; Gujral et al., 2012; Jabri and Sollid, 2009; Taylor et al., 1993).

Activated by gluten, CD4+ T-helper (Th) cells also respond by expressing high levels of interleukins 21, 2, 4, 6, and 15 (IL-21, IL-2, IL-4, IL-6, and IL-15) and interferon gamma (IFNG). These cytokines lead to Th cell–driven antibody responses, and they activate intraepithelial lymphocytes (Kaukinen et al., 2014; Rush and Waechter, 1987; Trynka et al., 2010; Withoff et al., 2016).

## II. Human disease pathways

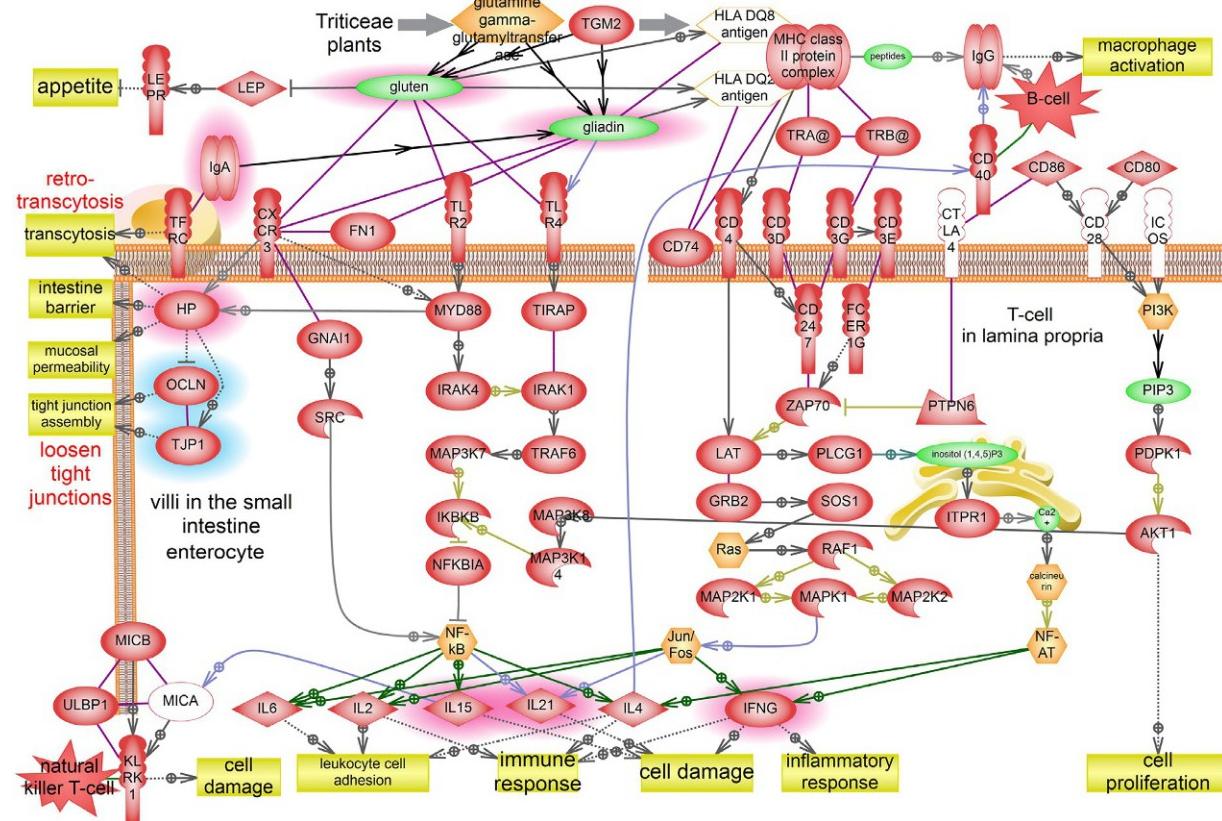


FIG. 9 Pathway 1: CD4+ T cell and epithelial cell response in celiac disease.

## Pathway 2

### CD8+ T-cell response and mucosal damage in celiac disease (Fig. 10)

#### Incoming signals

Intestinal epithelial cells express high levels of *IL-15*, together with MICs and HLA-E. IL-15 leads to the activation of KLRK1 signaling intraepithelial cytotoxic T lymphocytes and the resultant cytotoxic signaling pathway associated with this receptor.

#### Outcome effects

IL-15-induced perforin/granzyme and KLRK1–MICA signaling pathways promote the cytotoxic effects of intraepithelial lymphocytes on intestinal mucosa.

#### Signaling

Activation of NK cells through KLRK1 induces the phosphorylation of vav guanine nucleotide exchange factor 1 (VAV1) resulting in the actin-dependent clustering of stimulatory receptors and their recruitment to lipid rafts. That in turn leads to additional VAV1 phosphorylation and induces a positive feedback loop, which ultimately results in the release of arachidonic acids by intraepithelial cytotoxic T lymphocytes. They, in turn, can promote activation of intestinal granulocytes and inflammation.

The activation of NK cells also depends on the activation of stimulatory NK cell receptors. Integrin subunit alpha L (ITGAL) and integrin subunit beta 2 (ITGB2) can rapidly induce signals for activation by promoting adhesion between NK cells and a target cell. Fc fragment of IgG receptor IIIa (FCGR3A) and Fc fragment of IgE receptor Ig (FCER1G) provide antibody-dependent T cell–mediated cytotoxicity. FCGR3A and FCER1G recognize and bind to the Fc region of IgG, which is bound to the surface of a target cell. The stimulatory receptors of NK cells cause the release of calcium, and they activate the JUN/FOS transcriptional complex. NK cell–mediated responses include the transcription of cytokines and Fas ligand (FASLG) in addition to the formation of immunological synapses with granules of both perforins (PRF1) and granzymes (GZMA/GZMB). FASLG, PRF1, and GZMA/GZMB all result in the death of target cells. Cytokine-mediated signal transducer and activator of transcription 5 (STAT5) activation also results in the production of PRF1 and GZMA/GZMB and their consequent lethal effect on target cells (Kaukinen et al., 2014; Plenge, 2010; Trynka et al., 2010; Withoff et al., 2016).

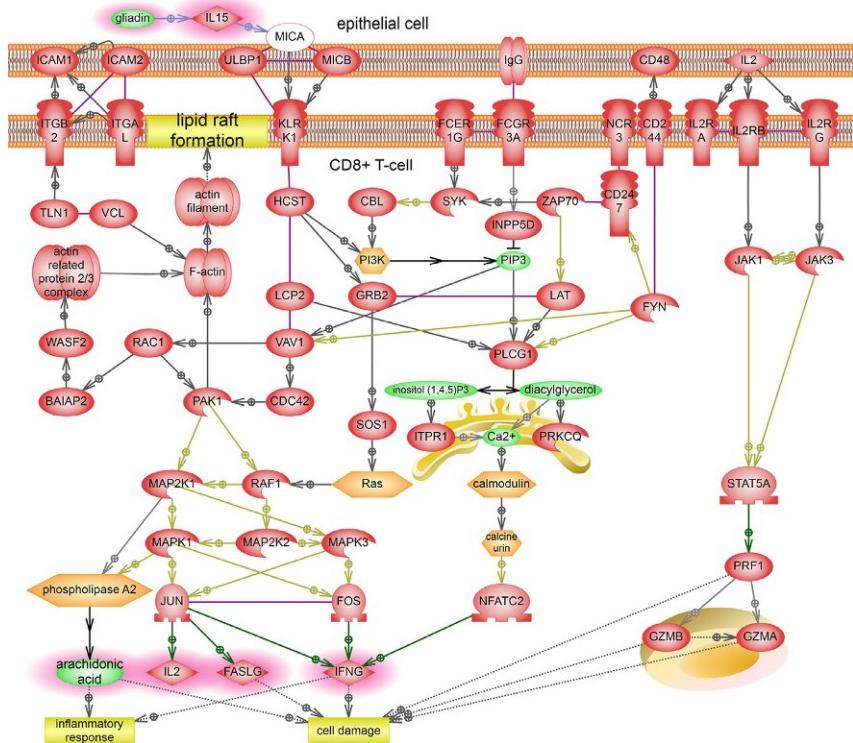


FIG. 10 Pathway 2: CD8+ T-cell response and mucosal damage in celiac disease.

## References

- Disease number #212750, #609754, and others Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code K90.0. Diseases of the digestive system (K00-K93). (ICD-10, <https://icdlist.com>). ICD-11: disease code DA95.
- Fasano, A., 2006. Systemic autoimmune disorders in celiac disease. *Curr. Opin. Gastroenterol.* 22, 674–679. <https://doi.org/10.1097/01.mog.0000245543.72537.9e>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Gujral, N., Freeman, H.J., Thomson, A.B.R., 2012. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J. Gastroenterol.* 18, 6036–6059. <https://doi.org/10.3748/wjg.v18.i42.6036>.
- Jabri, B., Sollid, L.M., 2009. Tissue-mediated control of immunopathology in coeliac disease. *Nat. Rev. Immunol.* 9, 858–870. <https://doi.org/10.1038/nri2670>.
- Kaukinen, K., Lindfors, K., Mäki, M., 2014. Advances in the treatment of coeliac disease: an immunopathogenic perspective. *Nat. Rev. Gastroenterol. Hepatol.* 11, 36–44. <https://doi.org/10.1038/nrgastro.2013.141>.
- Plenge, R.M., 2010. Unlocking the pathogenesis of celiac disease. *Nat. Genet.* 42, 281–282. <https://doi.org/10.1038/ng0410-281>.
- Rush, J.S., Waechter, C.J., 1987. Inhibitors of protein kinase C block activation of B lymphocytes by bacterial lipopolysaccharide. *Biochem. Biophys. Res. Commun.* 145, 1315–1320.
- Taylor, A.K., Lebwohl, B., Snyder, C.L., Green, P.H., 1993. Celiac disease. In: Adam, M.P., Ardingier, H.H., Pagon, R.A., Wallace, S.E., Bean, L.J., Mefford, H.C., Stephens, K., Amemiya, A., Ledbetter, N. (Eds.), GeneReviews®. University of Washington, Seattle, Seattle, WA.
- Trynka, G., Wijmenga, C., van Heel, D.A., 2010. A genetic perspective on coeliac disease. *Trends Mol. Med.* 16, 537–550. <https://doi.org/10.1016/j.molmed.2010.09.003>.
- Withoff, S., Li, Y., Jonkers, I., Wijmenga, C., 2016. Understanding celiac disease by genomics. *Trends Genet.* 32, 295–308. <https://doi.org/10.1016/j.tig.2016.02.003>.

## CHAPTER

## 10.4

## Nonalcoholic fatty liver disease

The incidence of NAFLD is increasing worldwide cooperatively with obesity, hyperlipidemia (hypertriglyceridemia), insulin resistance, and metabolic syndrome. However, the molecular mechanisms underlying NAFLD development are not yet understood.

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of diseases occurring in patients who do not abuse alcohol. (*Ferri and Ferri, 2018*).

NAFLD is a widespread disorder that affects up to 30% of the general population. Patients with NAFLD usually have no symptoms, but some of them may feel abdominal pain or discomfort, fatigue, and malaise.

It was shown that NAFLD is associated with an inactivating mutation in the *PNPLA3* gene. The *PNPLA3* gene codes an adiponutrin protein involved in the production and degradation of lipids (lipogenesis and lipolysis, respectively). Expression of the *PNPLA3* gene is enhanced after food intake and diminishes during fasting periods. *PNPLA3* gene variations lead to increased lipogenesis and decreased lipolysis in both adipocytes and hepatocytes, and they are related to the risk of NAFLD development (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

NAFLD is a range of disorders. Excessive lipid accumulation in the liver causes steatosis.

**Pathway 1. Triglyceride storage in NAFLD (Fig. 11).**

Over time, excess lipids can become toxic for hepatocytes and trigger inflammation and nonalcoholic steatohepatitis (NASH) leading to liver injury.

**Pathway 2. Lipotoxicity in NAFLD (Fig. 12).**

About 20% of patients with NAFLD suffer steatohepatitis. Recovery from insignificant damage is possible, but more severe damage can lead to the substitution of normal liver tissue with connective tissue (fibrosis) resulting in irreversible liver cirrhosis. People with NASH and cirrhosis have an increased risk of hepatocellular cancer development.

## Key cellular contributors and processes

Fatty acid beta oxidation

Cell process

Beta oxidation of fatty acids is the catabolic process in which the fatty acyl chain is broken down to acetyl-CoA molecules.

Glycogenesis

Cell process

Glycogenesis is the process of glycogen formation from glucose.

Glycolysis

Cell process

Glycolysis is the catabolic process that converts glucose to pyruvate.

Hepatocytes

Cell

Hepatocytes are the main cell type of the liver parenchyma and occupy approximately 80% of the liver volume. Hepatocytes are responsible for the major liver functions including detoxification, protein synthesis, and metabolism of lipids and carbohydrates.

Hyperlipidemia

Pathological condition

Hyperlipidemia is the condition with the excessive levels of triglycerides and cholesterol in the blood that is usually asymptomatic state but can trigger a number of diseases.

Insulin resistance

Pathological condition

Insulin resistance leads to an excess concentration of insulin in blood (hyperinsulinemia), as pancreatic beta cells produce more insulin in response to hyperglycemia caused by the inability of the cells to respond to insulin.

## Pathway 1

### Triglyceride storage in NAFLD (Fig. 11)

#### Incoming signals

Lipids are involved in many essential cellular processes. Derivatives of lipids and fatty acids (palmitic acid, stearic acid, and oleic acid) in triglycerides (TGs) are stored in cytoplasmic lipid droplets and used as a source of energy. Under physiological conditions, insulin adjusts the interrelation between carbohydrate and lipid metabolism. It controls fatty acid synthesis from glucose and the subsequent conversion into TGs. A common clinical feature of patients with NAFLD is impaired lipid metabolism associated with obesity, insulin resistance (IR), and hyperinsulinemia. The term “selective insulin resistance” is used to describe the metabolic events within the liver in NAFLD (Kawano and Cohen, 2013). Under the conditions of insulin resistance and hyperinsulinemia, glucose metabolism is disturbed in the liver, but the stimulation of lipogenesis by insulin continues. IR in the visceral adipose tissue intensifies lipolysis and increases the supply of adipose-derived free fatty acids (FFAs) to the liver. Hyperinsulinemia causes the imbalance between lipid synthesis and transformation and leads to lipid accumulation in the hepatocytes.

#### Outcome effects

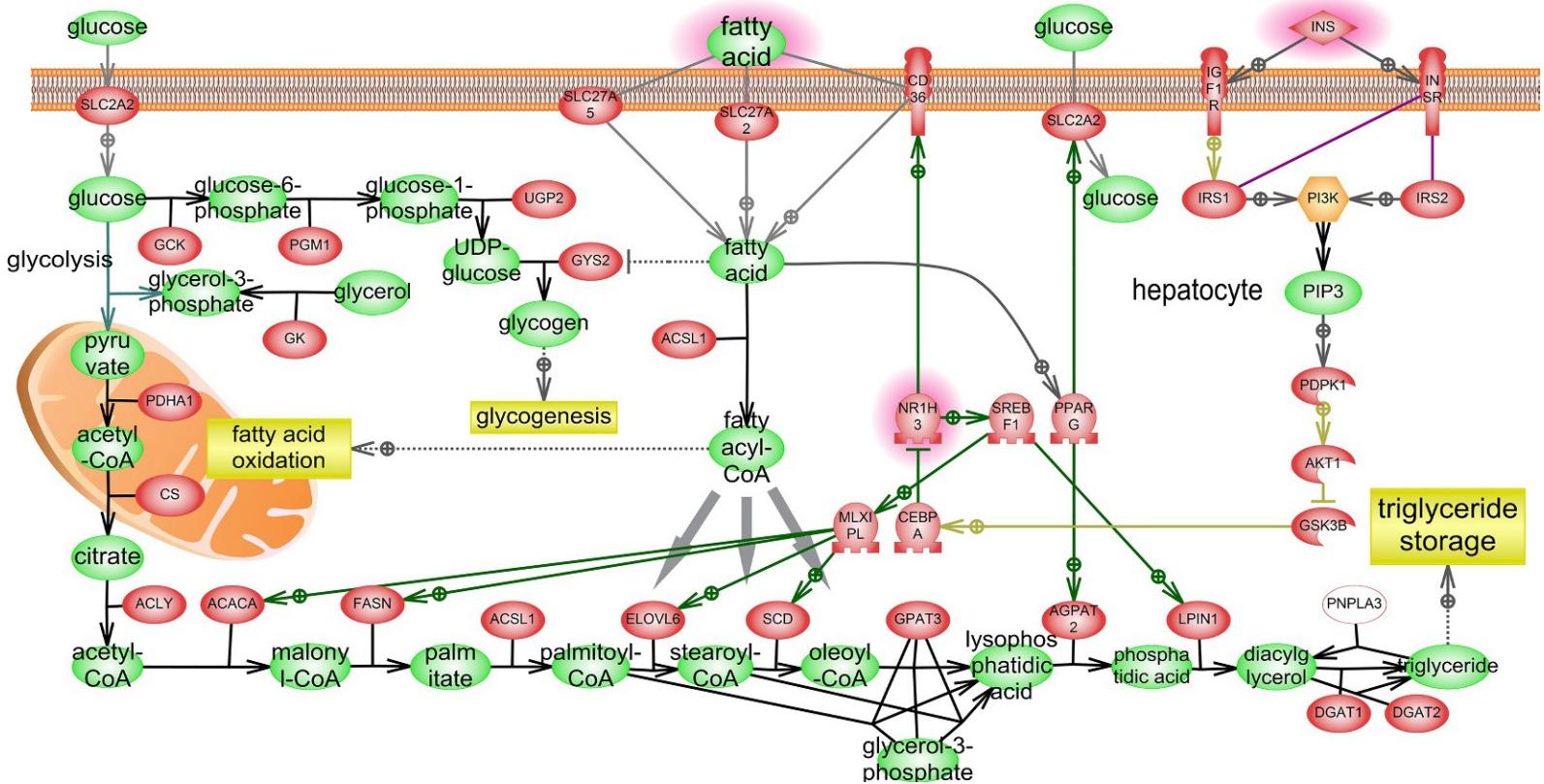
TGs accumulate in cytoplasmic lipid droplets forming steatosis. Mitochondria in hepatocytes try to adapt to increased FFAs by enhancing their beta-oxidation rate (the metabolic process of fatty acids degradation). However, increased transport of beta oxidation-derived products leads to increased reactive oxygen species (ROS) release. Excessive ROS release causes oxidative stress (not shown) leading to mitochondrial impairment and further hepatocyte dysfunction.

#### Signaling

FFAs are taken up by the membrane fatty acid transporters SLC27A2, A5, and translocase CD36. Once in the cell, FFAs are activated to fatty acid acyl-CoA molecules such as palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA by acyl-CoA synthetase (ACSL1). Fatty acid acyl-CoA participates in TGs synthesis. Glucose is transported by the glucose transporter SLC2A2 and undergoes glycolysis (universal metabolic process of glucose oxidation) and glycogenesis (formation of glucose reserves in the form of glycogen in the cells). Pyruvate, produced by glycolysis, is used for the synthesis

of acetyl-CoA, a lipogenesis substrate. Acetyl-CoA carboxylase (ACACA) then converts acetyl-CoA into malonyl-CoA. Fatty acid synthase (FASN) catalyzes palmitate formation from malonyl-CoA. There is then a sequential synthesis of palmitoyl-CoA, stearoyl-CoA, and oleoyl-CoA (fatty acids acyl-CoA) by acyl-CoA synthetase (ACSL1), fatty acid elongase (ELOVL6), and stearoyl-CoA desaturase (SCD), respectively. Lysophosphatidic acid acyltransferases (GPAT3 and AGPAT2) catalyze the conversion of glycerol-3-phosphate and fatty acid acyl-CoA to lysophosphatidic acid and further to phosphatidic acid. Lipin 1 (LPIN1) hydrolyzes phosphatidic acid into diacylglycerol. Diacylglycerol O-acyltransferases 1,2 (DGAT1,2) catalyzes the final step, which is the conversion of diacylglycerol into triglycerides. PNPLA3 hydrolyzes TGs into diacylglycerols in hepatocytes and prevents TGs storage. TGs can be stored within hepatic lipid droplets or secreted into the blood as very-low-density lipoproteins (VLDL).

High fatty acid levels lead to the induction of transcription factor PPARG. Insulin (INS) stimulates INSR/PI3K/AKT1 signaling, which in turn activates the transcription factors NR1H3, SREBF1, and MLXIPL. Increased levels of NR1H3 expression in the liver has been observed in NAFLD ([Higuchi et al., 2008](#)). NR1H3 induces CD36. PPARG, SREBF1, and MLXIPL enhance the production of SLC2A2 and the lipogenic enzymes ACACA, FASN, ACSL1, SCD, AGPAT2, and LPIN1. FFAs inhibit glycogen synthase GYS2, causing a reduction in glycogenesis and the redirection of glucose toward lipogenesis. Thus an excess of FFAs and “selective insulin resistance” together stimulate lipogenesis in the liver and provoke TG storage ([Berlanga et al., 2014; Ipsen et al., 2018; Kawano and Cohen, 2013; Pappachan et al., 2017; Vacca et al., 2015](#)).



**FIG. 11** Pathway 1: Triglyceride storage in NAFLD.

## Pathway 2

### Lipotoxicity in NAFLD (Fig. 12)

#### Incoming signals

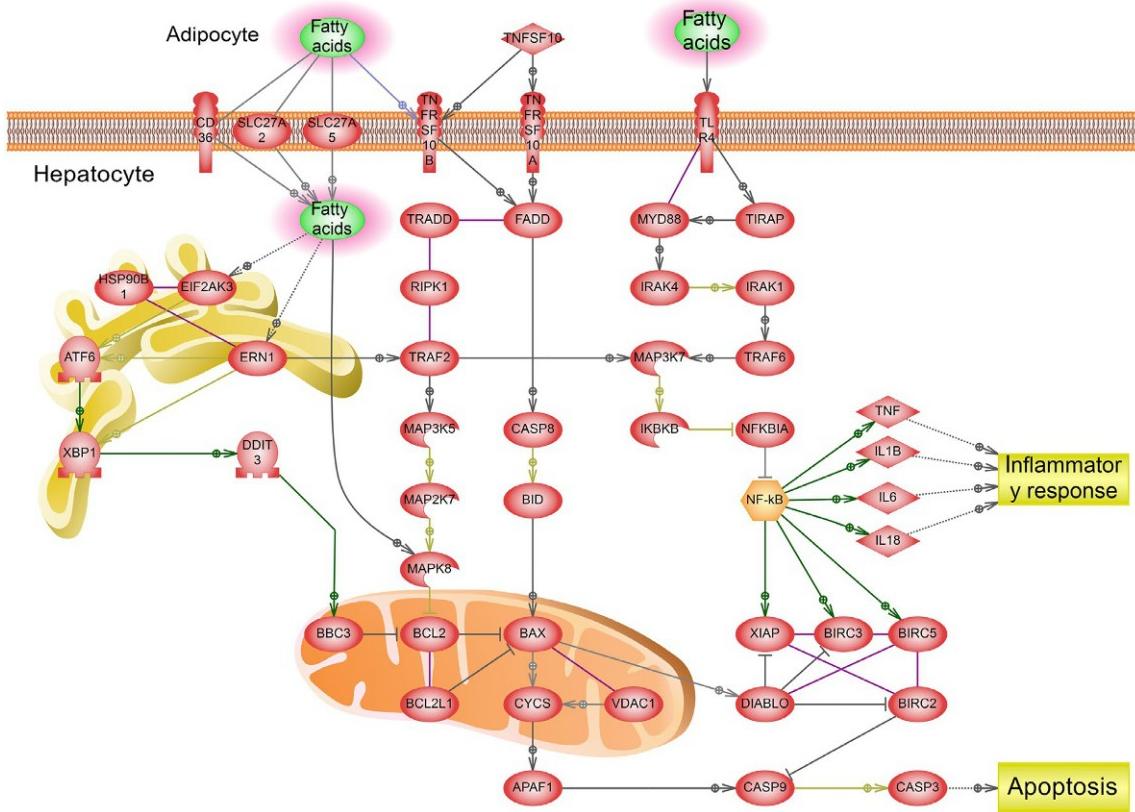
Excess FFAs can be toxic for hepatocytes. Hepatocytes take up FFAs and usually process and store them as triglycerides (TGs). But when hepatic FFAs exceed the accumulation quota, they activate hepatocyte apoptosis and inflammation.

#### Outcome effects

Hepatocyte apoptosis and inflammation are crucial mechanisms that promote progression of steatosis in steatohepatitis. Dead hepatocytes are consumed by macrophages leading to the release of proinflammatory signals. These stimuli along with proinflammatory cytokines produced by hepatocytes activate the hepatic stellate cells involved in liver fibrosis.

#### Signaling

FFAs contribute death receptor TNFRSF10B clustering resulting in the activation of caspase 8 via the adaptor protein FADD. Active CASP8 cleaves the cell death regulator protein BID, which is a member of the BCL2 family of proteins. The BCL2 family of cell death regulators plays a central role in the life-or-death choice in cellular fate. The BCL2 family includes both anti- and proapoptotic members, and the balance between their activities regulates the release of apoptosis-promoting proteins such as cytochrome *c* (CYCS), DIABLO, and BBC3. CYCS is released from mitochondria and binds to the apoptotic activating factor APAF1, which in turn mediates the processing of procaspase 9, which itself activates the main apoptosis regulator caspase 3. DIABLO suppresses BIRC, the caspase 9 inhibitor. The death receptors TNFRSF10B,10A stimulate MAPK-signaling through the signal transducers TRADD and TRAF2 to inactivate the anti-apoptotic protein BCL2. Pathological elevation of FFAs induces endoplasmic reticulum (ER) stress. FFAs activate the ER stress initiators EIF2AK3 and ERN1, which interact with the transcription factors ATF6 and XBP1 and BBC3. FFAs also modulate inflammation through TLR4. TLR4 activates IRAK4,1/MAP3K7/NFKB signaling that promotes the production of the proinflammatory cytokines IL-1B, IL-6, IL-18, and TNF involved in the inflammatory response (Akazawa and Nakao, 2016; Alkhouri et al., 2009; Lebeaupin et al., 2018; Takaki et al., 2014).



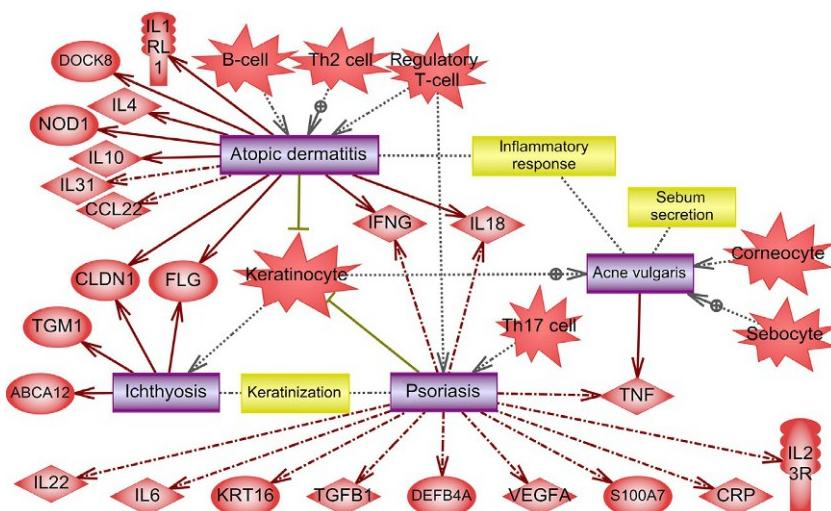
**FIG. 12** Pathway 2: Lipotoxicity in NAFLD.

## References

- Disease number #613282, #613387, and others Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code K76.0. K76.0 Fatty (change of) liver, not elsewhere classified. Diseases of the digestive system (K00-K93). (ICD-10, <https://icdlist.com>). ICD-11: disease code DB92.
- Akazawa, Y., Nakao, K., 2016. Lipotoxicity pathways intersect in hepatocytes: endoplasmic reticulum stress, c-Jun N-terminal kinase-1, and death receptors. *Hepatol. Res. Off. J. Jpn. Soc. Hepatol.* 46, 977–984. <https://doi.org/10.1111/hepr.12658>.
- Alkhouri, N., Dixon, L.J., Feldstein, A.E., 2009. Lipotoxicity in nonalcoholic fatty liver disease: not all lipids are created equal. *Expert Rev. Gastroenterol. Hepatol.* 3, 445–451. <https://doi.org/10.1586/egh.09.32>.
- Berlanga, A., Guiu-Jurado, E., Porras, J.A., Auguet, T., 2014. Molecular pathways in non-alcoholic fatty liver disease. *Clin. Exp. Gastroenterol.* 7, 221–239. <https://doi.org/10.2147/CEG.S62831>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Higuchi, N., Kato, M., Shundo, Y., Tajiri, H., Tanaka, M., Yamashita, N., Kohjima, M., Kotoh, K., Nakamura, M., Takayanagi, R., Enjoji, M., 2008. Liver X receptor in co-operation with SREBP-1c is a major lipid synthesis regulator in nonalcoholic fatty liver disease. *Hepatol. Res. Off. J. Jpn. Soc. Hepatol.* 38, 1122–1129. <https://doi.org/10.1111/j.1872-034X.2008.00382.x>.
- Ipsen, D.H., Lykkesfeldt, J., Tveden-Nyborg, P., 2018. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell. Mol. Life Sci.* <https://doi.org/10.1007/s00018-018-2860-6>.
- Kawano, Y., Cohen, D.E., 2013. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. *J. Gastroenterol.* 48, 434–441. <https://doi.org/10.1007/s00535-013-0758-5>.
- Lebeaupin, C., Vallée, D., Hazari, Y., Hetz, C., Chevet, E., Bailly-Maitre, B., 2018. Endoplasmic reticulum stress signaling and the pathogenesis of non-alcoholic fatty liver disease. *J. Hepatol.* <https://doi.org/10.1016/j.jhep.2018.06.008>.
- Pappachan, J.M., Babu, S., Krishnan, B., Ravindran, N.C., 2017. Non-alcoholic fatty liver disease: a clinical update. *J. Clin. Transl. Hepatol.* 5, 384–393. <https://doi.org/10.14218/JCTH.2017.00013>.
- Takaki, A., Kawai, D., Yamamoto, K., 2014. Molecular mechanisms and new treatment strategies for non-alcoholic steatohepatitis (NASH). *Int. J. Mol. Sci.* 15, 7352–7379. <https://doi.org/10.3390/ijms15057352>.
- Vacca, M., Allison, M., Griffin, J.L., Vidal-Puig, A., 2015. Fatty acid and glucose sensors in hepatic lipid metabolism: implications in NAFLD. *Semin. Liver Dis.* 35, 250–261. <https://doi.org/10.1055/s-0035-1562945>.

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# Diseases of the skin and subcutaneous tissue



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Diseases of the skin and subcutaneous tissue vary from minor rashes or skin defects to severe pathologies. Infections, exposure to allergens, and environmental pressure are the main causes of dermatological problems. Further, genetic factors and systemic immune or inflammatory reactions can be involved in or predispose to skin pathologies. Many skin diseases are chronic and not curable, but the symptoms can be successfully treated.

Acne vulgaris is one the most widespread dermatological diseases that can affect otherwise healthy people and induce strong psychological discomfort.

The most common skin conditions are various types of dermatitis or chronic inflammatory or immunological reactions in the skin. Dermatitis involves rashes, pain, and skin injuries. This chapter includes common chronic and severe forms of dermatitis—atopic dermatitis and psoriasis. Both conditions are characterized by the activation of autoimmune reactions involving overactive T-helper cells and share common symptoms. Atopic dermatitis and psoriasis have complex underlying genetic factors along with vague and unclear predispositions seen in different populations.

In contrast, ichthyosis is a group of hereditary disorders caused by known single-gene mutations and characterized by the formation of dry and thick skin. Ichthyosis is described in this chapter to emphasize the differences in our knowledge of pathogenic mechanisms underlying the disease.

## CHAPTER

## 11.1

## Acne vulgaris

The enlargement of sebaceous glands is one of the major processes underlying acne vulgaris. This pathological enlargement is a result of increased sebocyte proliferation.

Acne lesions can be divided into two types based on their appearance: inflammatory or noninflammatory.

Acne vulgaris is a chronic disorder of the pilosebaceous apparatus. It is caused by abnormal desquamation of follicular epithelium which leads to obstruction of the pilosebaceous canal. Such obstruction results in inflammation and formation of papules, pustules, nodules, comedones, and scarring. (*Ferrari, 2017, p. 1*).

Noninflammatory lesions are characterized by open and closed comedones. For inflammatory acne, lesions can be described by the presence of papules, pustules, and nodules (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Acne is the most common skin disease. About 85% of all teenagers are affected to some degree by acne, although children and adults may also be affected.

Risk factors for acne development have not been completely identified. However, contributors include infections, hormones, diet, and stress. *Propionibacterium acnes*, androgens, growth hormone, insulin-like growth factor 1, high glycemic-load diet, and excessive milk consumption may contribute to the development of acne. The genetic susceptibility to acne is probably due to the effects of numerous genes.

In the progression of inflammation-induced acne, the bacterium *P. acnes* triggers the synthesis of proinflammatory proteins in keratinocytes:

**Pathway 1.** *Inflammation in keratinocytes in the pathology of acne* (Fig. 1). An active inflammatory process at the site of acne lesions causes the formation of purulence (not shown) and a further stimulation of fatty acid production and sebaceous gland enlargement.

**Pathway 2.** *Increased proliferation of sebocytes in acne vulgaris* (Fig. 2).

**Pathway 3.** *Hyperseborrhea in acne vulgaris* (Fig. 3).

## Key cellular contributors and processes

Hyperseborrhea

Process

Hyperseborrhea is an excessive production of sebum by sebaceous glands. It can cause acne formation, as the thick sebum provides a breeding ground for *P. acnes* bacteria that participate in acne pathogenesis.

Inflammasome

Anatomic structure

The inflammasome is a multiprotein complex of the innate immune response system. The inflammasome activates the expression of proinflammatory interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-18 (IL-18) and promotes inflammation. Dysregulation of inflammasome function is involved in the pathogenesis in a variety of autoimmune diseases.

Sebocyte

Cell

Sebocytes are cells that constitute the sebaceous glands and produce sebum (a mixture of fatty acids and other molecules). Sebocytes participate in the pathological processes that take place in the sebaceous gland, for example, acne formation.

Toll-like receptors

Protein or gene

Toll-like receptors belong to a family of membrane proteins, which can directly bind microbial molecules or proteins and initiate the innate immune response.

## Pathway 1

### Inflammation in keratinocytes in the pathology of acne (Fig. 1)

#### Incoming signals

The characteristic inflammatory reaction in acne vulgaris is caused by the bacterium *P. acnes* that activates Toll-like receptor (TLR2 and TLR4) signaling resulting in the synthesis of proinflammatory cytokines such as interleukin-1 beta (IL-1B) and tumor necrosis factor (TNF).

#### Outcome effects

Proinflammatory cytokines are highly expressed in acne lesions, and they stimulate the activation of mast cells, neutrophils, monocytes, and other cells of the immune system. The inflammatory reaction supported by immune cells causes redness and pain and the formation of purulence in acne lesion.

#### Signaling

*P. acnes* provokes TLR2/TLR4 signaling and activation of the inflammasome. The inflammasome is a protein complex that promotes caspase-1 (CASP1) activity that in turn cleaves pro-IL-1B to activate it.

TNF and IL-1B play an important role in the inflammatory reaction in acne. They bind to their respective receptors on the cell surface and mediate the inflammatory response through mitogen-activated protein kinase, or MAPK signaling. Induction of transcription factor complex JUN/FOS leads to degradation of the extracellular matrix (ECM) around acne lesions by matrix metalloproteinases (MMPs). In addition, activation of the universal transcriptional factor NF- $\kappa$ B causes increased expression of proinflammatory proteins including IL-8, IL-1B, beta-defensin 4A (DEFB4A), IL-12A, IL-12B, and TNF itself. TNF and the interleukins promote neutrophil recruitment in the foci of infection (Bergler-Czop, 2014; Bhambri et al., 2009; Tanghetti, 2013).

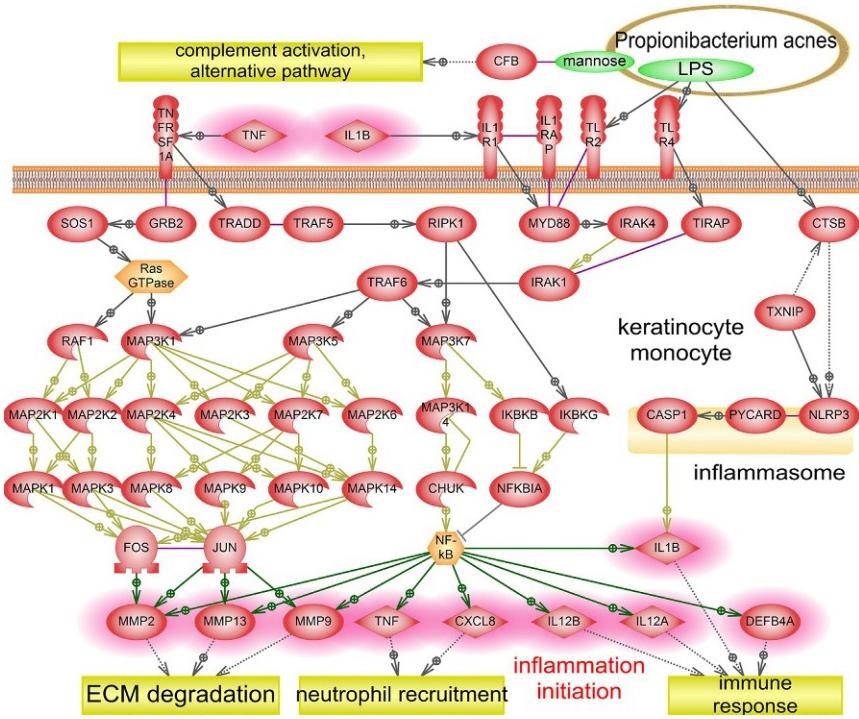


FIG. 1 Pathway 1: Inflammation in keratinocytes in the pathology of acne.

## Pathway 2

### Increased proliferation of sebocytes in acne vulgaris (Fig. 2)

#### Incoming signals

Various factors stimulate sebocyte proliferation and provoke enlargement of the sebaceous gland. Among them are proinflammatory proteins, milk consumption, and hormones. Another inducer of this process is reactive oxygen species (ROS). *P. acnes* also contributes to this process by producing free fatty acids and activating the nuclear transcription factors PPARA and PPARG.

#### Outcome effects

The amplified proliferation of sebocytes causes growth of the sebaceous glands and is one of the main reasons for the formation of lesions in acne vulgaris.

#### Signaling

Milk consumption and growth hormone (GH1) can stimulate insulin-like growth factor (IGF1) and insulin (INS) expression in sebocytes. IGF1 and INS bind to their respective receptors to activate the MAPK signaling. The downstream signal of the activated MAPK signaling initiates expression of cyclin D1 (CCND1), a key regulator of cell proliferation.

Fibroblast growth factors (FGF7 or FGF10) and proopiomelanocortin (POMC) affect the same signaling pathway.

A reduced amount of linoleic acid (which is itself an inhibitor of oxidative stress and ROS formation) was observed in patients with acne. Glucose-6-phosphate dehydrogenase (G6PD) and catalase (CAT) are usually involved in the reduction of ROS formation; however, these enzymes were shown to be downregulated in acne vulgaris. ROS interact with membrane lipids and cause the formation of cytotoxic lipid peroxides, or they oxidize squalene. This leads to the upregulation of arachidonate 5-lipoxygenase (ALOX5) activity, which, along with leukotriene A4 hydrolase (LTA4H), converts arachidonic acid into leukotriene B4. Lipid peroxides and leukotriene B4 can promote sebocyte proliferation via activation of the nuclear transcription factors PPARA and PPARG. These transcription factors are also upregulated by free fatty acids that enter the cell. Not only can the excessive amounts of free fatty acids come with food consumption, but also they may also be formed by a lipoprotein lipase of the Gram-positive anaerobic bacterium *P. acnes* (Bergler-Czop, 2014; Bhambri et al., 2009; Zouboulis, 2004).

II. Human disease pathways

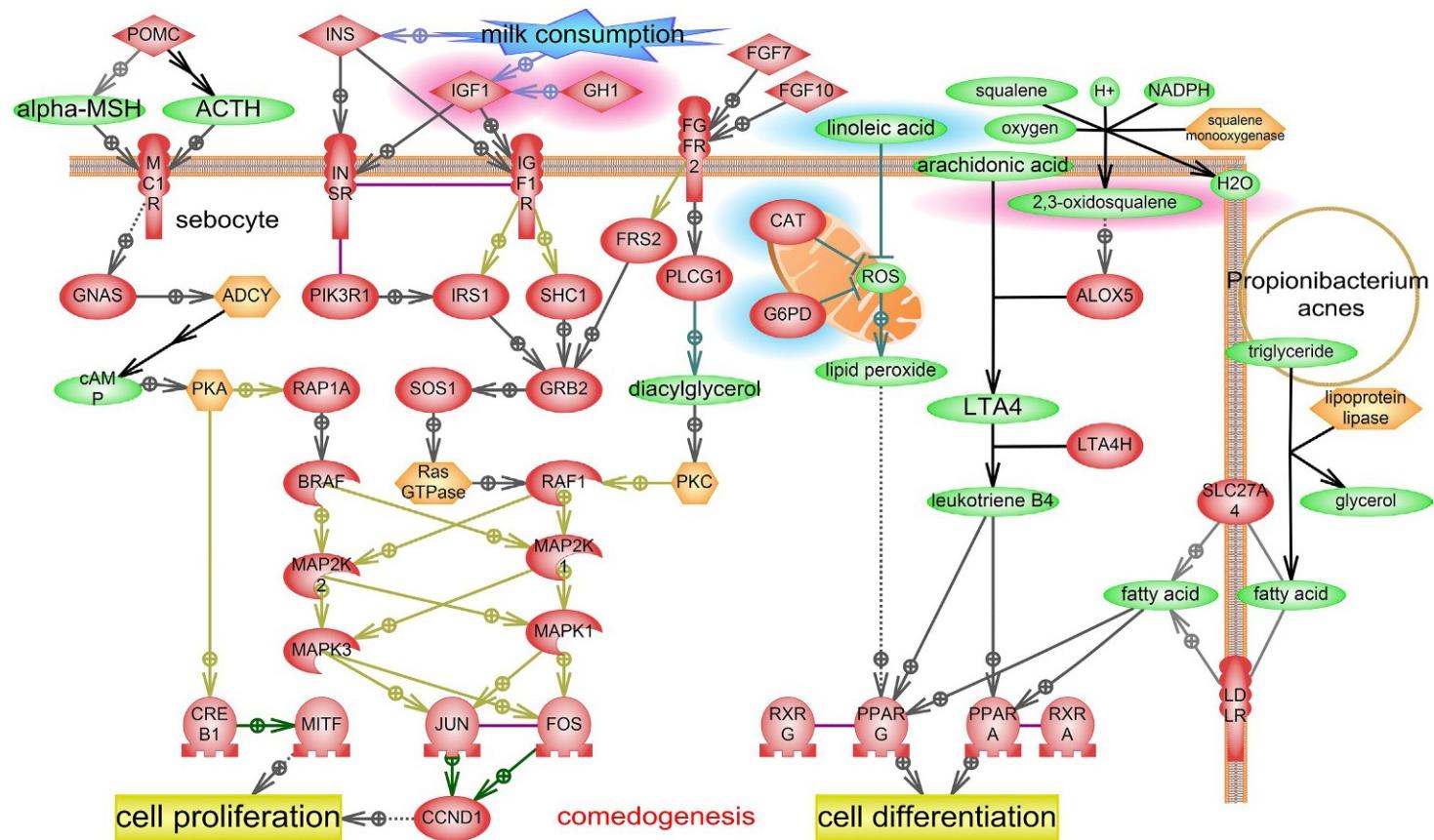


FIG. 2 Pathway 2: Increased proliferation of sebocytes in acne vulgaris.

## Pathway 3

### Hyperseborrhea in acne vulgaris (Fig. 3)

#### Incoming signals

Hyperseborrhea, or the excessive production of sebum by sebaceous glands, results from increased fatty acid biosynthesis in sebocytes. Hyperseborrhea may be induced by various factors including IGF1, cytokines, and free fatty acids themselves. Milk consumption and growth hormone (GH1) expression (not shown) can influence the synthesis and secretion of IGF1 in the liver. Testosterone also increases sebocyte hyperfunction.

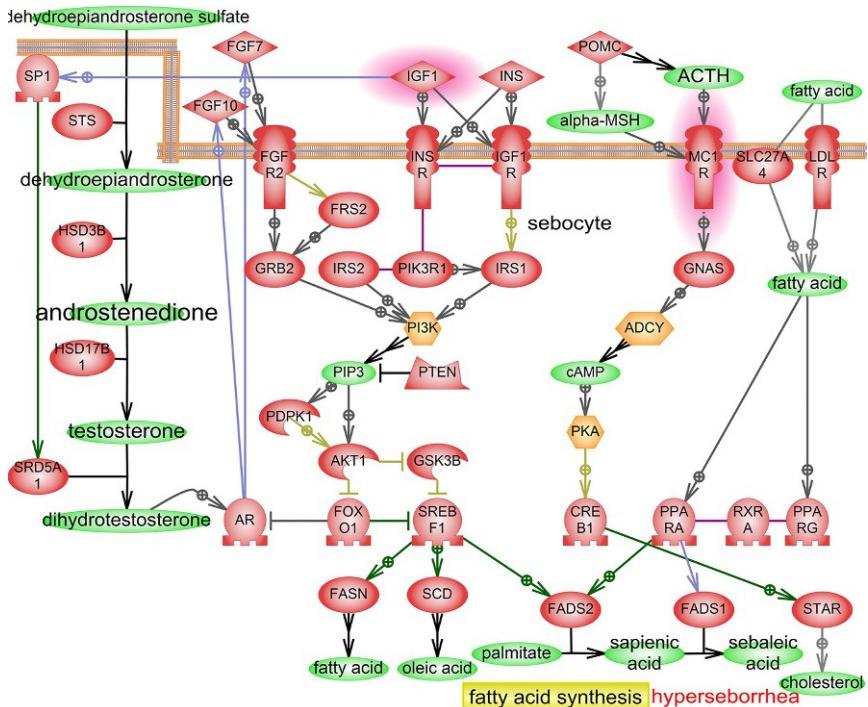
#### Outcome effects

Increased fatty acid biosynthesis by sebocytes and increased sebocyte proliferation lead to comedogenesis and hyperseborrhea: hyperseborrhea results in inflammation and abnormal keratinocyte function at the site that can cause the formation of acne lesions.

#### Signaling

Increased fatty acid biosynthesis in sebocytes can be provoked by the following factors: insulin-like growth factor (IGF1), insulin (INS), fibroblast growth factors (FGF7,10), proopiomelanocortin (POMC), and free fatty acids. IGF1 and other proteins activate PI3K/AKT1 signaling. AKT1-mediated phosphorylation of the nuclear transcription factor forkhead box protein O1 (FOXO1) leads to the relief of sterol regulatory element-binding transcription factor 1 (SREBF1) and promotes fatty acid biosynthesis. In addition, increased expression of the melanocortin 1 receptor (MC1R) is also observed in sebocytes associated with acne vulgaris. POMC binds to MC1R to activate the transcription factor CREB1 and induce cholesterol synthesis.

Free fatty acids contribute to the observed increased levels of lipogenesis in sebocytes following their translocation via low-density lipoprotein receptor (LDLR) and the fatty acid transporter SLC27A4 into the cell. PPARG and PPARA are transcription factors that promote fatty acid biosynthesis, and they are stimulated by fatty acids too. In the sebocyte the circulating androgenic prohormone, dehydroepiandrosterone (DHEA), is converted to dihydrotestosterone. Both testosterone and dihydrotestosterone are able to bind to the androgen receptor (AR). However, dihydrotestosterone has a 10-fold higher affinity to AR compared with testosterone. Dihydrotestosterone exerts its effects on sebaceous glands by promoting sebocyte proliferation mediated by the transcription of androgen-responsive genes (not shown) and by elevating lipid production via SREBF1 (Bowe and Logan, 2010; Youn, 2010; Zouboulis, 2004).



**FIG. 3** Pathway 3: Hyperseborrhea in acne vulgaris.

## References

- Disease number # 604324 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code L70.0. Diseases of the skin and subcutaneous tissue (L00-L99). (ICD-10, <https://icdlist.com>). ICD-11: disease code ED80.Z.
- Bergler-Czop, B., 2014. The aetiopathogenesis of acne vulgaris—what's new? *Int. J. Cosmet. Sci.* 36, 187–194. <https://doi.org/10.1111/ics.12122>.
- Bhambri, S., Del Rosso, J.Q., Bhambri, A., 2009. Pathogenesis of acne vulgaris: recent advances. *J. Drugs Dermatol.* 8, 615–618.
- Bowe, W.P., Logan, A.C., 2010. Clinical implications of lipid peroxidation in acne vulgaris: old wine in new bottles. *Lipids Health Dis.* 9, 141. <https://doi.org/10.1186/1476-511X-9-141>.
- Ferri, F.F., 2017. Ferri's Clinical Advisor 2017. 5 Books in 1.
- Tanghetti, E.A., 2013. The role of inflammation in the pathology of acne. *J. Clin. Aesthetic Dermatol.* 6, 27–35.
- Youn, S.W., 2010. The role of facial sebum secretion in acne pathogenesis: facts and controversies. *Clin. Dermatol.* 28, 8–11. <https://doi.org/10.1016/j.cldermatol.2009.03.011>.
- Zouboulis, C.C., 2004. Acne and sebaceous gland function. *Clin. Dermatol.* 22, 360–366. <https://doi.org/10.1016/j.cldermatol.2004.03.004>.

## CHAPTER

## 11.2

## Atopic dermatitis

Atopic dermatitis is a disorder characterized by inflammation of the epidermis and dermis of the skin (dermatitis). Dry, itchy skin and red rashes that come and go are hallmarks of atopic dermatitis. The rashes can occur on any part of the body, although the pattern tends to be different at different ages (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Atopic dermatitis (AD) (atopic eczema, eczema) is a genetically determined eczematous eruption that is pruritic, symmetric, and associated with personal family history of allergic manifestations (atopy). (*Ferrari, 2017, p. 1*).

AD is characterized by typical clinical (erythema, pruritus, papules, vesicles, seizures, crusts, and lichenification) and dermatopathological (hyper- and parakeratosis, spongiosis, acanthosis, lymphocytic infiltrates, and exocytosis) symptoms. AD etiology is not yet completely understood, but it is clearly multifactorial. Complex interactions between the heredity and environmental factors, such as allergens or immunogens, skin barrier dysfunctions, and pruritus, are also very important for AD progression. Atopic dermatitis is a chronic skin disorder that typically involves a skin reaction, similar to that of an allergy. IgE-mediated sensitization to food and environmental allergens is one of the possible mechanisms leading to AD. The impaired skin barrier function allows irritants and allergens to penetrate the skin and cause inflammation via an overactive T-helper cells type 2 response (with increased IL-4 and IL-5 cytokines) in acute lesions (*Darsow et al., 2014; Kolb and Ferrer-Bruker, 2017; Otsuka et al., 2017*). Currently, AD can be defined as “a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis, or eczema/dermatitis” (*Johansson et al., 2004*). The highest incidence of atopic dermatitis is observed among children, and they account for 10%–20% of the population.

After contact with an antigen such as *Staphylococcus aureus*, cutaneous professional antigen-presenting cells (such as Langerhans cells) migrate to regional lymph nodes where they present processed peptides

to naive T cells. Activated naive T lymphocytes then differentiate toward a Th2 phenotype and induce IgE production by B lymphocytes:

**Pathway 1.** *The onset of atopic dermatitis* (Fig. 4).

The acute phase, which is characterized by eczematous skin lesions, is facilitated by allergen/IgE-bound Langerhans cells (LCs) and by IgE-activated mast cells.

**Pathway 2.** *Acute phase initiation in atopic dermatitis* (Fig. 5).

## Key cellular contributors and processes

Chemokines

Protein or gene

Chemokines are a family of a larger group of extracellular signaling molecules called cytokines. Chemokines are secreted low-molecular-weight proteins that can induce chemotaxis-directed movement of a cell in response to a molecular stimulus.

IgE-mediated sensitization

Process

In allergy the immune response starts with allergic sensitization that can be described as follows. Upon allergen encounter an antigen-presenting cell (APC) presents the allergen to a T cell that is then differentiated into a Th2 cell, which in turn promotes differentiation of a B cell into an allergen-specific immunoglobulin E antibody (IgE)-producing plasma cell. Further the allergen-specific IgE binds to the surface of mast cell and basophils, which can then recognize the allergen (are sensitized to the allergen). The next time the allergen enters the body, it can bind to the IgE molecules on the surface of the mast cells and basophils, and an allergic reaction to the introduced allergen might develop.

Immunoglobulin E antibodies

Protein or gene

Immunoglobulin E (IgE) antibodies are a type of antibodies produced by plasma B cells in response to allergens and parasites. IgE monomers consist of two heavy chains and two light chains. IgE antibodies have an essential role in allergic immune response.

Langerhans cells

Cell

Langerhans cells (LCs) are antigen-presenting cells (dendritic cells) of the epidermis. LCs contain a specific type of organelles known as the Birbeck granules found exclusively in these cells.

Major histocompatibility complex class II

Protein or gene

The major histocompatibility complex (MHC) class II is a heterodimeric protein complex on the surface of antigen-presenting cells. The MHC class II molecules have a fundamental role in processing extracellular antigens and presenting them to T cells.

## Proinflammatory cytokines

### Protein or gene

Cytokines are a broad list of small proteins released by immune cells that participate in cell-to-cell communication and regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFNG), granulocyte-macrophage colony-stimulating factor (GMCS-F), and others), secreted primarily by macrophages and T-helper cells, upregulate proinflammatory reactions.

## Type 1 and Type 2 T-helper cells

### Cell

Type 1 and Type 2 T-helper cell (Th1, Th2 cell) are cells of the T-cell lineage that protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) via stimulation of B-cell maturation and activation of other immune cells.

## Pathway 1

### The onset of atopic dermatitis ([Fig. 4](#))

#### Incoming signals

Allergens derived from microbes and foods may enter the skin in areas with defective barrier functions to be picked up by Langerhans cells (LCs), inflammatory dendritic epidermal cells, and plasmacytoid dendritic cells. Mature LCs express high levels of major histocompatibility complex (MHC) class II antigens, costimulatory molecules such as CD80 and CD86, and chemokine receptors, which are all important for antigen presentation. Antigens combined with MHC class II and other signals from activated Langerhans cells and dendritic cells contribute to the subsequent processes underlying atopic dermatitis such as the recruitment of inflammatory cells, T-cell priming, and the release of cytokines and chemokines by mature T cells.

#### Outcome effects

Mature Th2 cells in turn promote differentiation of B cells into IgE-producing plasma cells (not shown). Furthermore, AD is associated with increased IgE levels and decreased IFNG production.

#### Signaling

Antigen-loaded LCs migrate to lymph nodes and stimulate naive CD4+ T cells (Th0) to differentiate into Th2 cells. High levels of CD80 and CD86 cause the constitutive activation of the AKT1 and MAPK-signaling cascade that leads to Th2 cell proliferation.

On the other hand, high levels of MHC class II antigens cause the activation of the T-cell receptor (TCR) signaling in naive T cells. Src family lymphocyte-specific protein tyrosine kinase Lck (LCK) is activated after the TCR signaling stimulation. LCK then phosphorylates CD3 coreceptor cytoplasmic domains in the TCR complex. These phosphorylated domains (immunoreceptor tyrosine-based activation (ITAM)) serve as binding sites for zeta chain-associated protein kinase (ZAP70). Activated ZAP70 then phosphorylates a variety of linker/adapter proteins such as the linker responsible for the activation of T-cell (LAT) family and LCP2. The further recruitment of downstream proteins results in calcium mobilization, stimulation of the MAPK cascade, reorganization of the actin cytoskeleton, and activation of the Ras small monomeric GTPases. Enzymatically active transducers, such as PI3K and phospholipase C gamma (PLCG1), help relay signals downstream. These signals lead to the activation of nuclear transcription factors such as JUN, FOS, NFAT, and NF- $\kappa$ B that ultimately result in elevated expression of the cytokines IL-2, IL-4, IL-5, and IFNG to trigger the Th2 cell immune response and cell proliferation ([Darsow et al., 2014; Islam and Luster, 2012; Liu et al., 2011](#)).

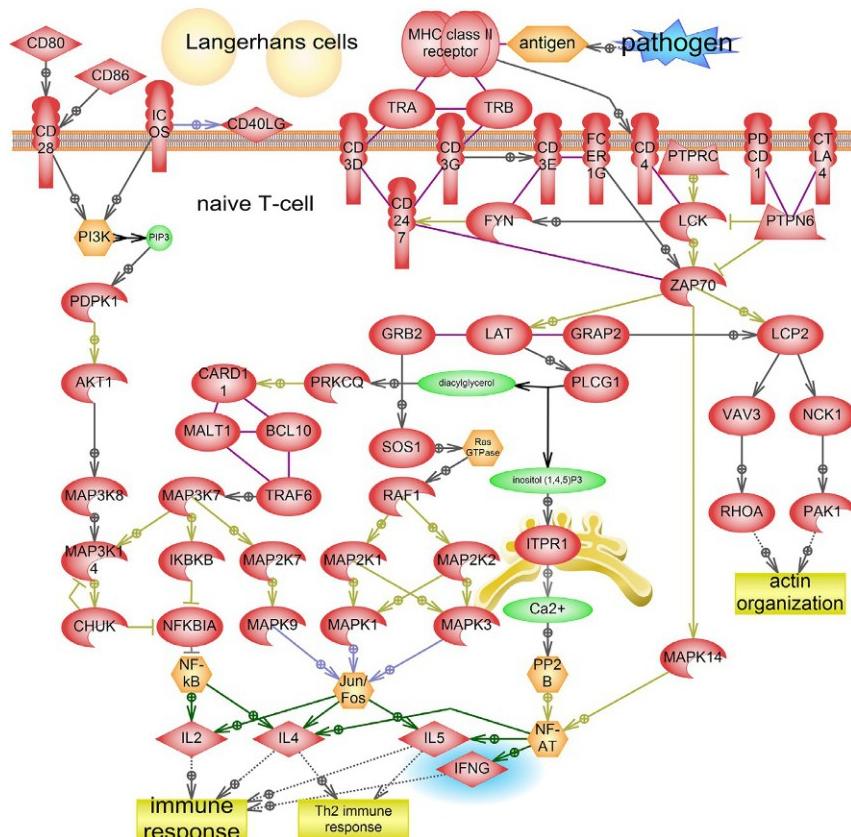


FIG. 4 Pathway 1: The onset of atopic dermatitis.

## Pathway 2

### Acute phase initiation in atopic dermatitis (Fig. 5)

#### Incoming signals

The acute phase of AD, which is characterized by eczematous skin lesions, is facilitated by allergen/IgE-bound Langerhans cells (LCs) and by infiltration of skin-homing Th2 cells.

After contact with an antigen such as *S. aureus*, professional antigen-presenting cells in the skin migrate to regional lymph nodes where they present processed antigens to naive T cells. Naive T cells, in turn, promote the differentiation of Th2 cells, which then promote the differentiation of B cells into IgE-producing plasma cells. Additional allergens are picked up by IgE receptors in antigen-specific IgE-bound Langerhans cells. More antigen-loaded LCs then migrate to lymph nodes and stimulate native CD4+ T cells to differentiate and generate a new wave of IgE expression by B cells.

Circulating IgE binds to high-affinity IgE receptors on skin-resident mast cells to sensitize them. Mast cells in AD serve as the major effector cells in immediate hypersensitivity through activation via the high-affinity IgE receptor (FCER1). FCER1-activated mast cells secrete the broad array of proinflammatory mediators involved in the sensitization to allergens and IgE elevation.

Besides, allergens, bacteria, mechanical injury, and exogenous proteases induce the release of IL-33 and thymic stromal lymphopoietin (TLSP) from keratinocytes. IL-33, alone or in combination with other cytokines and chemokines, activates mast cells in inflamed skin.

#### Outcome effects

Eczematous skin lesions develop as a result of the complex immune and inflammatory responses driven by the release of proinflammatory cytokines and chemokines from various skin-resident cell types. IgE sensitizes mast cells in the skin, and activated Th2 cells produce IL-4, IL-5, IL-13, and other proteins, which reinforce the Th2-type reaction as follows: IL-4 and IL-13 bind to receptors on keratinocytes and suppress filaggrin production. IL-4 promotes the production of IgE by B cells; IL-31 provokes pruritus. IL-4 and IL-13 released by mast cells contribute to the migration of Th2 cells into the involved site. Chemokines and IL-5 induce the infiltration of eosinophils across venules.

## Signaling

IgE-dependent activation of mast cells involves the binding of IgE to several FCERI receptors. Each FCERI receptor consists of four subunits: an alpha particle that is responsible for IgE binding, an intracellular beta subunit, and two intracellular gamma subunits. Antigen binding to IgE results in the cross-linking of two FCERI receptors, which leads to the phosphorylation of tyrosine kinase SYK. SYK then activates a multiprotein complex that includes GRB2 and VAV1 formed on LAT. SYK activates proteins that in turn induce the downstream MAPK-cascade and a calcium mobilization pathway, which leads to the synthesis of IL-4, IL-13, CXCL8, and TNF. IL-33 also can induce the release of IL-13 independently from IgE stimulation.

Th2 cells, through activation of the T-cell receptor (TCR) signaling, activate nuclear transcription factors such as JUN, FOS, NFAT, and NF- $\kappa$ B that ultimately result in the next level maturation of immune cells: B-cell activation, eosinophil function, and isotype switching. IL-4 is the main Th2-released cytokine, and it is simultaneously the activator of Th2 cell itself. Th2 cells require IL-4 and its downstream effector signal transducer and activator of transcription 6 (STAT6) for development. STAT6 participates in Th2 differentiation, in part by enhancing the expression of the GATA3 gene, which encodes the master regulators of Th2 differentiation. These factors, together with IL-2-mediated STAT5 activation, induce the secretion of copious amounts of interleukin by Th2 cell.

The intersection of signaling pathways initiated by IgE and IL-31 leads to eosinophil migration, the Th2 immune response, and B-cell activation ([Cevikbas and Steinhoff, 2012](#); [Darsow et al., 2014](#); [Gittler et al., 2013](#); [Islam and Luster, 2012](#); [Liu et al., 2011](#); [Otsuka et al., 2017](#)).

## II. Human disease pathways

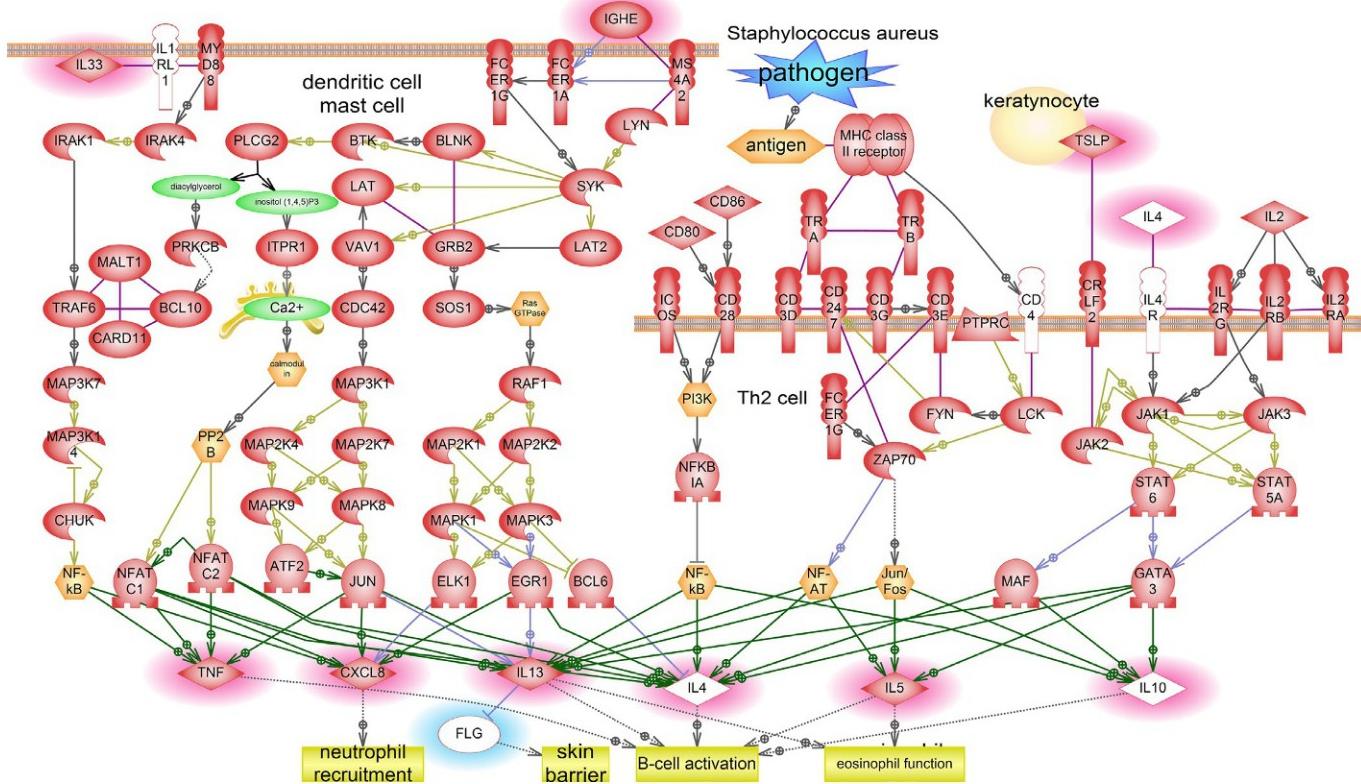


FIG. 5 Pathway 2: Acute phase initiation in atopic dermatitis.

## References

- Disease number # 603165 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code L20.0. Diseases of the skin and subcutaneous tissue (L00-L99). (ICD-10, <https://icdlist.com>). ICD-11: disease code EA80.
- Cevikbas, F., Steinhoff, M., 2012. IL-33: a novel danger signal system in atopic dermatitis. *J. Invest. Dermatol.* 132, 1326–1329. <https://doi.org/10.1038/jid.2012.66>.
- Darsow, U., Raap, U., Ständer, S., 2014. Atopic dermatitis. In: Carstens, E., Akiyama, T. (Eds.), *Itch: Mechanisms and Treatment*. Frontiers in NeuroscienceCRC Press/Taylor & Francis, Boca Raton, FL.
- Ferri, F.F., 2017. Ferri's Clinical Advisor 2017. 5 Books in 1.
- Gittler, J.K., Krueger, J.G., Guttmann-Yassky, E., 2013. Atopic dermatitis results in intrinsic barrier and immune abnormalities: implications for contact dermatitis. *J. Allergy Clin. Immunol.* 131, 300–313. <https://doi.org/10.1016/j.jaci.2012.06.048>.
- Islam, S.A., Luster, A.D., 2012. T cell homing to epithelial barriers in allergic disease. *Nat. Med.* 18, 705–715. <https://doi.org/10.1038/nm.2760>.
- Johansson, S.G.O., Bieber, T., Dahl, R., Friedmann, P.S., Lanier, B.Q., Lockey, R.F., Motala, C., Ortega Martell, J.A., Platts-Mills, T.A.E., Ring, J., Thien, F., Van Cauwenberge, P., Williams, H.C., 2004. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.* 113, 832–836. <https://doi.org/10.1016/j.jaci.2003.12.591>.
- Kolb, L., Ferrer-Bruker, S.J., 2017. Dermatitis, atopic. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Liu, F.-T., Goodarzi, H., Chen, H.-Y., 2011. IgE, mast cells, and eosinophils in atopic dermatitis. *Clin. Rev. Allergy Immunol.* 41, 298–310. <https://doi.org/10.1007/s12016-011-8252-4>.
- Otsuka, A., Nomura, T., Kerknimit, P., Seidel, J.A., Honda, T., Kabashima, K., 2017. The interplay between genetic and environmental factors in the pathogenesis of atopic dermatitis. *Immunol. Rev.* 278, 246–262. <https://doi.org/10.1111/imr.12545>.

## CHAPTER

## 11.3

## Psoriasis

Psoriasis is a chronic autoimmune skin disorder characterized by scaly and itchy patches of red skin. It is generally considered to be a hereditary disease triggered by environmental factors. Most of the genetic changes associated with psoriasis relate to the immune system.

Psoriasis is a chronic skin disorder characterized by excessive proliferation of keratinocytes, resulting in the formation of thickened scaly plaques, itching, and inflammatory changes of the epidermis and dermis. The various forms of psoriasis include guttate, pustular, and arthritis variants. (*Ferri, 2017, p. 1*).

The pathogenesis of psoriasis has yet to be fully understood. Studies have shown that dysregulation of T cells resident in the skin is involved in the development of psoriasis plaques (Girolomoni et al., 2012, p. 17; Lowes et al., 2008). Psoriasis affects 1%–3% of the world's population. The peak of disease manifestation occurs during adolescence. About 85% of psoriasis patients have mild-to-moderate disease involving <5% of their body.

Psoriasis skin lesions contain increased amounts of T-helper 17 (Th17) cells. Genetic mutations and environmental factors together act to dysregulate the differentiation of the skin T cells and induce a shift to Th17 cell production:

**Pathway 1. Differentiation of psoriatic T cells (Fig. 6).**

IL-17 and IL-22 promote the proliferation of keratinocytes, a key sign of psoriasis. Keratinocytes synthesize a wide range of proinflammatory cytokines, which attract neutrophils, activate dendritic cells, and maintain the inflammatory reaction in the skin.

**Pathway 2. Interleukin-17 and interleukin-22 signaling in psoriasis (Fig. 7).**

## Key cellular contributors and processes

Chemokines

Protein or gene

Chemokines are a family of a larger group of extracellular signaling molecules called cytokines. Chemokines are secreted low-molecular-weight proteins, which can induce chemotaxis-directed movement of a cell in response to a molecular stimulus.

Cytokines

Protein or gene

Cytokines are a broad list of small proteins released by immune cells, which participate in cell-to-cell communication and regulate immune responses. Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors.

Hyperproliferation

Process

Hyperproliferation refers to an abnormally high rate of cell proliferation by quickened divisions.

Interleukins

Protein or gene

Interleukins are a subgroup of a large group of extracellular signaling molecules called cytokines. Interleukins are low-molecular-weight proteins involved in the functioning of both the adaptive and the innate immune system.

Th17 cells

Cell

Th17 cells are a subset of T-helper lineage cells, which preferentially express interleukin-17A, IL-17F, IL-21, and IL-22. The Th17 cells act as proinflammatory agents by recruiting neutrophils and macrophages to the infected site. Th17 cells are implicated in the development of autoimmune diseases.

Type 1 and Type 2 T-helper cells

Cell

Type 1 and Type 2 T-helper cell (Th1 and Th2 cell) are cells of the T-cell lineage that protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) via stimulation of B-cell maturation and activation of other immune cells.

## Pathway 1

### Differentiation of psoriatic T cells ([Fig. 6](#))

#### Incoming signals

The causes of psoriasis include genetic changes, environmental factors, and the dysregulation of the skin-resident T cells. Psoriatic skin lesions contain increased numbers of differentiated T-helper 17 (Th17) cells. The differentiation of naive T cells toward the Th17 phenotype is promoted by a number of cytokines. Those cytokines are transforming growth factor-B (TGFB1), IL-1B, IL-6, IL-18, IL-12 and IL-23, IFNA1 and IFNG, and others. A significant association between polymorphisms in the *IL-12B*, *IL-23A*, and *IL-23R* genes and patients with psoriasis had been shown.

#### Outcome effects

Increased number of Th17 cells expressing IL-23 and IL-17 are thought to be the specific regulators of the inflammatory reactions in psoriasis.

#### Signaling

Activated transforming growth factor beta receptor (TGFRB1) phosphorylates two downstream effectors, signal transducers, and transcriptional modulators SMAD2 and SMAD3, which leads to their translocation into the nucleus. The physical interaction between SMAD3 and the transcription factor STAT3 results in increased levels of expression of the *STAT3* gene. STAT3 is a key positive regulator of *RORC* gene expression, and it binds to the *IL-17* and *IL-21* promoters. Increased expression of *STAT3* and *RORC* is essential for Th17 cell development. The transcription factors STAT3 and RORC coordinate Th17 differentiation. STAT3 activation also reduces *GATA3* gene expression. GATA3 is an important regulator of Th2 cell development. Reduced activity of GATA3 leads to a decrease in the production of IL-10, IL-13, and IL-4.

Moreover, SMAD3 can reduce the activity of STAT4, an important regulator of Th1 cell development. Thus transforming growth factor beta (TGFB1) prevents Th1 and Th2 cell differentiation by suppressing STAT4 and GATA3 gene expression, allowing Th17 differentiation to occur instead. However, Th17 development also occurs in the absence of TGFB1 signaling. The depletion of FOXP3+ Treg cells and reduced Foxp3 mRNA levels are also observed in psoriasis patients. FOXP3 is a well-known regulator of Th17-cell differentiation and function.

The cooperative interaction between the IL-23, IL-12, IL-18, and IL-6 signaling pathways was also noted with augmented *STAT3* and *RORC*

gene expression. Interleukin receptors activate the Janus kinases 1 and 2 (JAK1 and JAK2) and tyrosine kinase 2 (TYK2) that subsequently phosphorylate and activate STAT3. In addition, IFNA1-stimulated psoriatic T cells show enhanced levels of JAK1 and TYK2. The action of IFNA1 on psoriatic T cells can be explained by the downregulation of protein tyrosine phosphatase (PTPN6) expression within them. PTPN6 regulates JAK1,2 phosphorylation and blocks the translocation of STATs into the nucleus. However, psoriatic T cells display low baseline expression levels of the PTPN6.

IL-1B negatively regulates Th1 cell differentiation by inducing the expression of prostaglandin synthase 2 (PTGS2), which subsequently leads to the secretion of prostaglandin E2 (PGE2) that suppresses IFNG production, thereby allowing Th17 cell differentiation via STAT3 activation ([Cai et al., 2012](#); [Eriksen et al., 2010](#); [Lowes et al., 2008](#); [Mudigonda et al., 2012](#); [Rubino et al., 2012](#); [Ruchusatsawat et al., 2006](#)).

## II. Human disease pathways

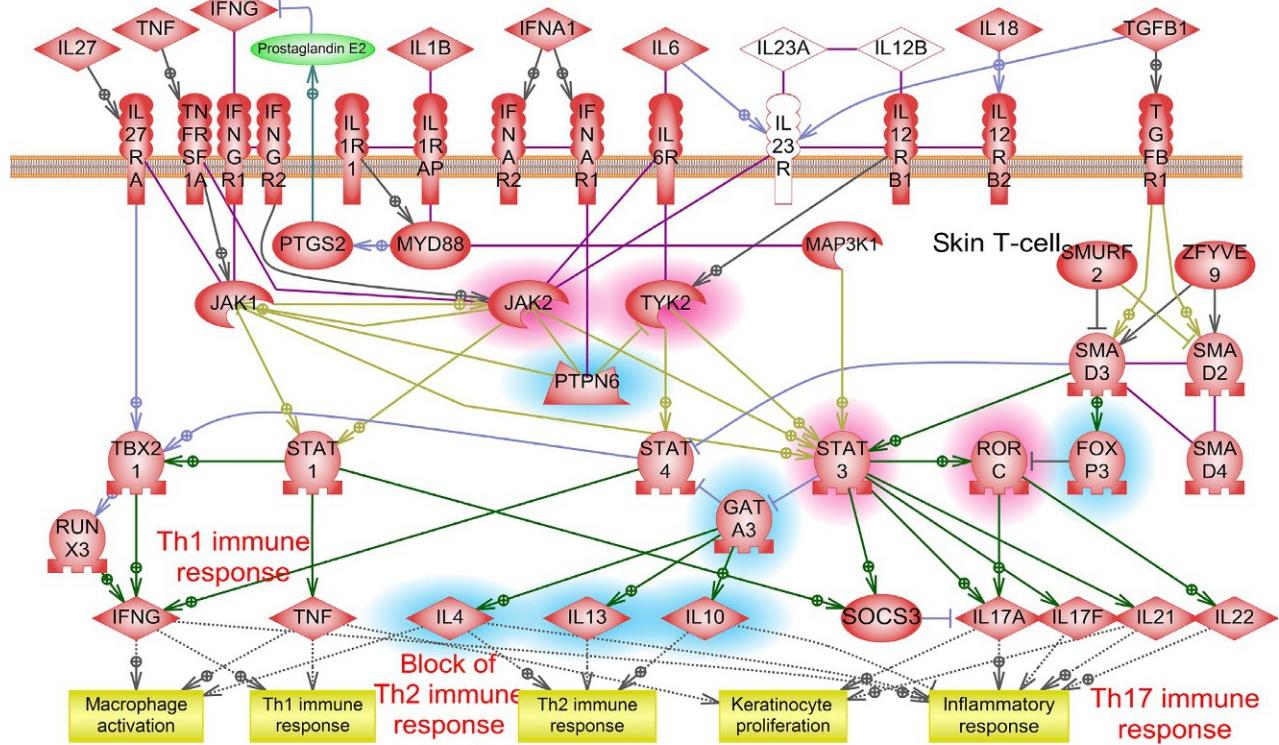


FIG. 6 Pathway 1: Differentiation of psoriatic T cells.

## Pathway 2

### Interleukin-17 and interleukin-22 signaling in psoriasis (Fig. 7)

#### Incoming signals

IL-17A and IL-17F are proinflammatory cytokines produced by activated Th17 cells involved in the pathogenesis of psoriasis. IL-22 is a cytokine that can significantly induce an increase in the proliferation of human keratinocytes.

#### Outcome effects

IL-17 stimulates the production of cytokines and chemokines, which are responsible for the recruitment of neutrophils to the site of inflammation. IL-22 signaling positively regulates keratinocyte proliferation.

Besides, IL-17 and IL-22 regulate the synthesis of several skin-specific proteins involved in the immune response including the defensins, psoriasin (S100A7), calgranulins (S100A8 and S100A9), keratin, desmocollin, and filaggrin.

In psoriasis, keratinocytes, which surround a wound, produce and release high levels of psoriasin and calgranulins in the wound exudate. The calgranulins interact with the receptor AGER and the toll-like receptors in cells of immune system within the skin, leading to increased expression of additional proinflammatory cytokines and proangiogenic factors (Gilliet and Lande, 2008; Schmidt et al., 2012; Sparvero et al., 2009).

#### Signaling

IL-17A and IL-17F activate NF- $\kappa$ B and the MAPK cascade through the adaptor protein TRAF3IP2. TRAF3IP2 further interacts with the interleukin signal transducer TRAF6 to promote signal transduction leading to the secretion of cytokines and chemokines such as CXCL1, CXCL2, CXCL5, CXCL8, CCL2, CCL7, and CCL20. In the NF- $\kappa$ B pathway, TRAF6 activates the inhibitor IKBKB in response to proinflammatory cytokines. Heterodimers of the interleukin receptors IL-17RA and IL-17RC recruit TRAF6 leading to the activation of the MAPK cascade. Furthermore the activity levels of MAPK1, MAPK3, MAPK14, and MAPK8 are increased in psoriatic skin (Girolomoni et al., 2012, p. 17; Madonna et al., 2010; Mudigonda et al., 2012).

IL-22 is produced by a specific subset of Th17 cells. It acts through the IL-22 receptor, which is a complex of two subunits, IL-22RA1 and IL-10RB. After binding to IL-22RA1, IL-22 activates Janus kinase 1 (JAK1), the signal transducer and activator of transcription STAT3, MAPKs, and

the PI3K/AKT1/MTOR signaling pathway. All this leads to an increase in the production of the matrix metallopeptidases 1, 9, 12 (MMP1, 9, 12), S100 calcium-binding proteins (S100A7, S100A8, and S100A9) and keratin 17 (KRT17).

*S100A7, A8, and A9* belong to a well-known psoriasis susceptibility locus (PSOR4) located on chromosome 1q21. Psoriasin and calgranulins are overexpressed in psoriasis. On the contrary a noticeable decrease in the expression levels of keratin 10 (KRT10), desmocollin 1 (DSC1), and filaggrin (FLG) were observed in psoriatic epidermal keratinocytes ([Hao, 2014](#); [Sestito et al., 2011](#)).

## II. Human disease pathways

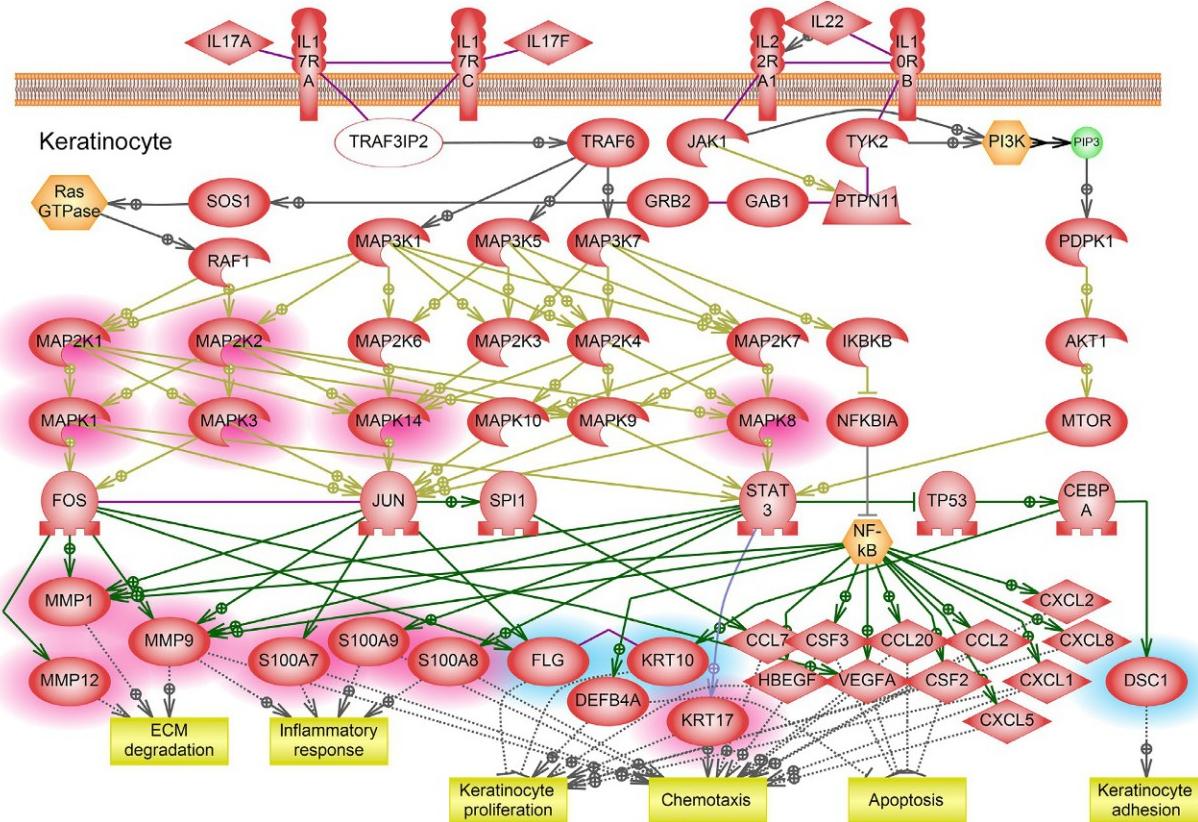


FIG. 7 Pathway 2: Interleukin-17 and interleukin-22 signalling in psoriasis

## References

- Disease number # 177900 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code L40. Diseases of the skin and subcutaneous tissue (L00-L99). (ICD-10, <https://icdlist.com>). ICD-11: disease code EA90/EA90.0.
- Cai, Y., Fleming, C., Yan, J., 2012. New insights of T cells in the pathogenesis of psoriasis. *Cell. Mol. Immunol.* 9, 302–309. <https://doi.org/10.1038/cmi.2012.15>.
- Eriksen, K.W., Woetmann, A., Skov, L., Krejsgaard, T., Bovin, L.F., Hansen, M.L., Grønbaek, K., Billestrup, N., Nissen, M.H., Geisler, C., Wasik, M.A., Ødum, N., 2010. Deficient SOCS3 and SHP-1 expression in psoriatic T cells. *J. Invest. Dermatol.* 130, 1590–1597. <https://doi.org/10.1038/jid.2010.6>.
- Ferri, F.F., 2017. Ferri's Clinical Advisor 2017. 5 Books in 1.
- Gilliet, M., Lande, R., 2008. Antimicrobial peptides and self-DNA in autoimmune skin inflammation. *Curr. Opin. Immunol.* 20, 401–407. <https://doi.org/10.1016/j.co.2008.06.008>.
- Girolomoni, G., Mrowietz, U., Paul, C., 2012. Psoriasis: rationale for targeting interleukin-17. *Br. J. Dermatol.* 167, 717–724. <https://doi.org/10.1111/j.1365-2135.2012.11099.x>.
- Hao, J.-Q., 2014. Targeting interleukin-22 in psoriasis. *Inflammation.* 37, 94–99. <https://doi.org/10.1007/s10753-013-9715-y>.
- Lowes, M.A., Kikuchi, T., Fuentes-Duculan, J., Cardinale, I., Zaba, L.C., Haider, A.S., Bowman, E.P., Krueger, J.G., 2008. Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J. Invest. Dermatol.* 128, 1207–1211. <https://doi.org/10.1038/sj.jid.5701213>.
- Madonna, S., Scarponi, C., Sestito, R., Pallotta, S., Cavani, A., Albanesi, C., 2010. The IFN-gamma-dependent suppressor of cytokine signaling 1 promoter activity is positively regulated by IFN regulatory factor-1 and Sp1 but repressed by growth factor independence-1b and Krüppel-like factor-4, and it is dysregulated in psoriatic keratinocytes. *J. Immunol.* 185, 2467–2481. <https://doi.org/10.4049/jimmunol.1001426>.
- Mudigonda, P., Mudigonda, T., Feneran, A.N., Alamdari, H.S., Sandoval, L., Feldman, S.R., 2012. Interleukin-23 and interleukin-17: importance in pathogenesis and therapy of psoriasis. *Dermatol. Online J.* 18, 1.
- Rubino, S.J., Geddes, K., Girardin, S.E., 2012. Innate IL-17 and IL-22 responses to enteric bacterial pathogens. *Trends Immunol.* 33, 112–118. <https://doi.org/10.1016/j.it.2012.01.003>.
- Ruchusatsawat, K., Wongpiyabovorn, J., Shuangshoti, S., Hirankarn, N., Mutirangura, A., 2006. SHP-1 promoter 2 methylation in normal epithelial tissues and demethylation in psoriasis. *J. Mol. Med.* 84, 175–182. <https://doi.org/10.1007/s00109-005-0020-6>.
- Schmidt, S.V., Nino-Castro, A.C., Schultze, J.L., 2012. Regulatory dendritic cells: there is more than just immune activation. *Front. Immunol.* 3, 274. <https://doi.org/10.3389/fimmu.2012.00274>.
- Sestito, R., Madonna, S., Scarponi, C., Cianfarani, F., Failla, C.M., Cavani, A., et al., 2011. STAT3-dependent effects of IL-22 in human keratinocytes are counterregulated by sirtuin 1 through a direct inhibition of STAT3 acetylation. *FASEB J.* 25, 916–927. <https://doi.org/10.1096/fj.10-172288>.
- Sparvero, L.J., Asafu-Adjei, D., Kang, R., Tang, D., Amin, N., Im, J., Rutledge, R., Lin, B., Amoscato, A.A., Zeh, H.J., Lotze, M.T., 2009. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. *J. Transl. Med.* 7, 17. <https://doi.org/10.1186/1479-5876-7-17>.

## CHAPTER

## 11.4

## Ichthyosis

Ichthyosis is a group of hereditary skin diseases. There are several clinical forms and a number of rare syndromes that include ichthyosis as one of their symptoms. Not all ichthyosis causes have yet been identified.

Ichthyosis is a family of genetic disorders with dry, scaly, and thickened skin.

Ichthyosis vulgaris is the mildest form of hereditary nonsyndromic ichthyosis, characterized by xerosis (abnormal skin dryness), eczema, pruritus, and atopic manifestations.

X-linked ichthyosis is the second most common ichthyosis, with a prevalence of 1:2000 to 1:6000. Recessive X-linked ichthyosis is clinically characterized by widespread, dark brown, polygonal scales and generalized dryness ([Takeichi and Akiyama, 2016](#)).

Harlequin ichthyosis is the most phenotypically severe inherited ichthyosis. Skin development is altered in utero. Babies with harlequin ichthyosis are born with very thick hard skin in form rhomb-shaped plates divided by deep cracks (fissures). These skin abnormalities deform the face and limit body movement (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Ichthyosis is one of the most frequent monogenic disorders in humans. It caused by mutations in one of several genes including the filaggrin gene (FLG):

**Pathway 1. Ichthyosis vulgaris overview (Fig. 8).**

X-linked ichthyosis is caused by mutations in the STS gene located on the X chromosome, which encodes steroid sulfatase.

**Pathway 2. X-linked ichthyosis overview (Fig. 9).**

Mutations in the gene encoding a member of the ABCA transporter family, ABCA12, have been linked to harlequin ichthyosis.

**Pathway 3. Harlequin ichthyosis overview (Fig. 10).**

## Key cellular contributors and processes

### Corneodesmosomes

#### Anatomic structure

Corneodesmosomes are specialized cell-cell adhesion structures that interconnect corneocytes, the major cell type in the stratum corneum. Corneodesmosomes maintain a strong epidermal sheet structure, and their disruption leads to desquamation.

### Lamellar granules

#### Anatomic structure

Lamellar granules (also called lamellar bodies, keratinosomes, Odland bodies, and membrane-coating granules MCGs) are membrane vesicles produced by skin keratinocytes or alveolar cells of the lung. In the skin, lamellar granules are secreted into the extracellular space between the epidermal layers and contain molecules, lipids, and proteins required for maintaining the lipid barrier and layered structure of the epidermis. In the lungs, lamellar bodies participate in the production of pulmonary surfactant.

### Lipid skin barrier

#### Process

The lipid skin barrier is a layer of lipids, ceramides, and fatty acids produced by keratinocytes in the stratum corneum layer of epidermis. The lipid matrix prevents excessive water loss through the epidermis and forms a physical barrier against harmful agents.

### Stratum corneum

#### Anatomic structure

Stratum corneum is the outer layer of the epidermis composed by dead corneocytes filled with keratin (skin cells) submerged in an intercellular matrix composed of lipids and fatty acids. Stratum corneum serves as a tough protective barrier for the inner layers of live cells.

### Stratum granulosum

#### Anatomic structure

Stratum granulosum (granular layer) is one of the intermediate layers of the epidermis localized between stratum corneum and stratum spinosum (although in thick skin, there is an additional layer just underneath stratum corneum called stratum lucidum). Granular layer keratinocytes contain dense lipid-rich granules called keratohyalin granules, which participate in the formation of hydrophobic barrier in the skin.

## Pathway 1

### Ichthyosis vulgaris overview (Fig. 8)

#### Incoming signals

Filaggrin (FLG) is a key protein of the keratohyalin granules in the stratum granulosum. Keratohyalin granules are important in the formation of the protective skin barrier and the terminal differentiation of the epidermis. FLG is associated with keratin intermediate filaments in the outer granular layer of the epidermis, and it supports their packing into bundles. During terminal keratinocyte differentiation, FLG is cross-linked to the “cornified cell envelope,” which constitutes an insoluble barrier in the stratum corneum against environmental agents. Filaggrin metabolites work as natural moisturizing factors, which are able to absorb a large amount of water, maintaining the necessary level of humidification of the tissue and the appropriate pH.

At least four loss-of-function mutations in FLG were significantly associated with ichthyosis vulgaris.

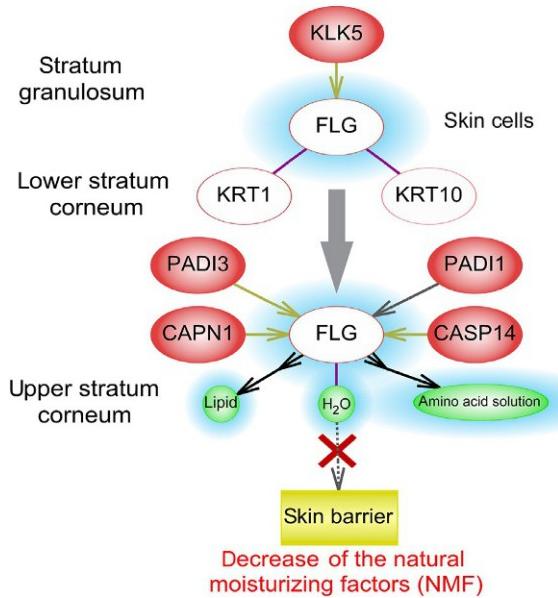
#### Outcome effects

Filaggrin is one of the main contributors to epidermal barrier integrity, and accordingly, filaggrin defects leads to the epidermal barrier dysfunctions.

#### Signaling

Profilaggrin is cleaved by several enzymes when it is converted to the functional filaggrin monomer protein. The profilaggrin linker domain is cleaved by the kallikrein-related peptidase 5KLK5. KLK5 is a major serine protease found in the skin, which is secreted from lamellar granules in the stratum granulosum and then activated in the extracellular space of the stratum corneum.

Filaggrin monomers can bind to keratin 1 or keratin 10 and to bundles of keratin intermediate filaments in the lower stratum corneum. Thus keratinocytes are flattened, and scales are formed on the lower stratum corneum. At the upper stratum corneum, filaggrin undergoes citrullination (the process in which arginine converts into citrulline) mediated by peptidyl arginine deiminase 1 and 3 (PADI1 and PADI3). Modified filaggrin is cleaved by calpain 1 (CAPN1), caspase-14 (CASP14), and other enzymes into free amino acids and their derivatives that together serve as natural moisturizing factors (NMF). NMF is a complex of low-molecular-weight substances including urocanic acid, pyrrolidone carboxylic acid, cyclic derivatives of glutamine, histidine metabolites, and citrulline (Hernández-Martin et al., 2013; Kezic and Jakasa, 2016; Takeichi and Akiyama, 2016; Thyssen et al., 2013).



**FIG. 8** Pathway 1: Ichthyosis vulgaris overview.

## Pathway 2

### X-linked ichthyosis overview (Fig. 9)

#### Incoming signals

Loss-of-function mutations in the gene encoding steroid sulfatase (*STS*) were found in patients with X-linked ichthyosis. *STS* hydrolyzes several 3-beta-hydroxysteroid sulfates, which are metabolic precursors of estrogens, androgens, and cholesterol. *STS* is ubiquitously expressed in tissues. Nonfunctional *STS* reduces the available cholesterol to form the extracellular lamellar bilayers in the skin. In patients with X-linked ichthyosis, the remodeling dynamics of corneodesmosome (an adhesion structure between living keratinocytes and the enucleated stratum corneum cells) is delayed. The formation of the peripheral, honeycomb pattern of corneodesmosome distribution is affected because corneodesmosome does not degrade properly. That disrupts the water-barrier function of the upper level of stratum corneum.

#### Outcome effects

Extra cholesterol sulfate in the stratum corneum destabilizes permeability barrier homeostasis by modifying the organization of the lamellar lipids and the ability of those lipids to inhibit corneodesmosome proteolysis. Cholesterol sulfate also drives epidermal differentiation and lipid synthesis. Reduced generation of cholesterol from cholesterol sulfate (reduced by approximately 50% in the disease) also contributes to the barrier abnormality (Kitajima, 2015).

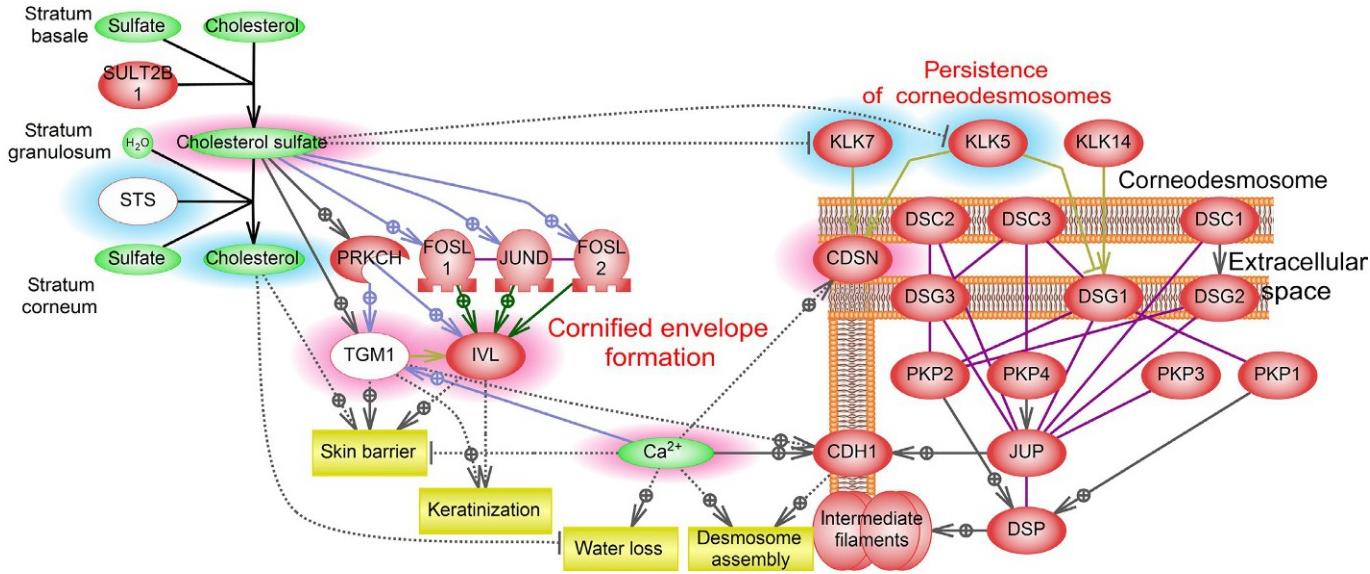
The stratum corneum of patients is rich in calcium along the corneodesmosomes.  $\text{Ca}^{2+}$ , if present in sufficient quantities, could stabilize the highly anionic sulfate groups (resulting from persistent cholesterol sulfate) in the extracellular lipids and form  $\text{Ca}^{2+}$  bridges. Formation of  $\text{Ca}^{2+}$  bridges between corneodesmosomes takes part in the delayed degradation of corneodesmosome observed in X-linked ichthyosis skin.

#### Signaling

Cytosolic sulfotransferase SULT2B1 catalyzes the conversion of cholesterol to cholesterol sulfate. *STS* desulfates cholesterol sulfate. SULT2B1 activity generates cholesterol sulfate predominately in the lower nucleated cell layers of the epidermis, while in contrast, *STS* levels peak in the outer epidermis and in sebocytes. Cholesterol sulfate increases the expression of the FOSL1, FOSL2, and JUND proteins, members of the AP-1 family of transcription factors, leading to enhanced synthesis of proteins that promote keratinization.

Cholesterol sulfate also may activate the eta isoform of protein kinase C (PRKCH), which in turn stimulates the phosphorylation of epidermal structural proteins and stimulates cornified envelope formation.

Cholesterol sulfate increases stratum corneum retention through inhibition of the serine proteases kallikreins 5 and 7 (KLK5 and KLK7), which are needed for the initial stages of corneodesmosome degradation. Corneodesmosomes mature from desmosomes and cannot reassemble once detached by degradation. Corneodesmosomes are connected directly to transmembrane desmosomal cadherins, desmogleins (DSGs), and desmocollins (DSCs), mainly DSG1/DSC1 with corneodesmosin. Corneodesmosome degradation is needed for the formation of more widespread lipid multilayers and wider intercellular space in the stratum corneum in order for the water-barrier function to be effective ([Elias et al., 2014](#); [Hernández-Martín et al., 1999](#); [Marukian and Choate, 2016](#); [Takeichi and Akiyama, 2016](#); [Wu and Paller, 2017](#)).



**FIG. 9** Pathway 2: X-linked ichthyosis overview.

## Pathway 3

### Harlequin ichthyosis overview (Fig. 10)

#### Incoming signals

Mutations in the gene encoding a member of the ABCA transporter family, ABCA12, have been linked to harlequin ichthyosis. The lack of ABCA12 function leads to the disruption of lamellar granule lipid transport in keratinocytes in the upper epidermis.

#### Outcome effects

The massive hyperkeratosis (stratum corneum thickening) that occurs in the patients with harlequin ichthyosis could be a compensatory response to a deficient lipid barrier.

#### Signaling

ABCA12 is essential for the transport of lipids and the regulation of protein synthesis in the developing skin layer. In the stratum corneum, ABCA12 transports glucosylceramides into epidermal lamellar bodies located in keratinocytes. Deficient ABCA12 function causes a decrease in intercellular lipid levels in the stratum corneum. *ABCA12* mutations have been associated with decreased levels of kallikrein-related peptidase 5 (KLK5) and cathepsin D (CTSD). ABCA12 is part of a feedback loop wherein increased amounts of cellular glucosylceramide and gangliosides accumulated in keratinocytes may induce their apoptosis.

Ceramide activates PPAR delta (PRARD), leading to the expression of the ABCA12 transmembrane transporter (Akiyama, 2014; Rodríguez-Pazos et al., 2013; Takeichi and Akiyama, 2016; Uchida, 2014; Zuo et al., 2008).

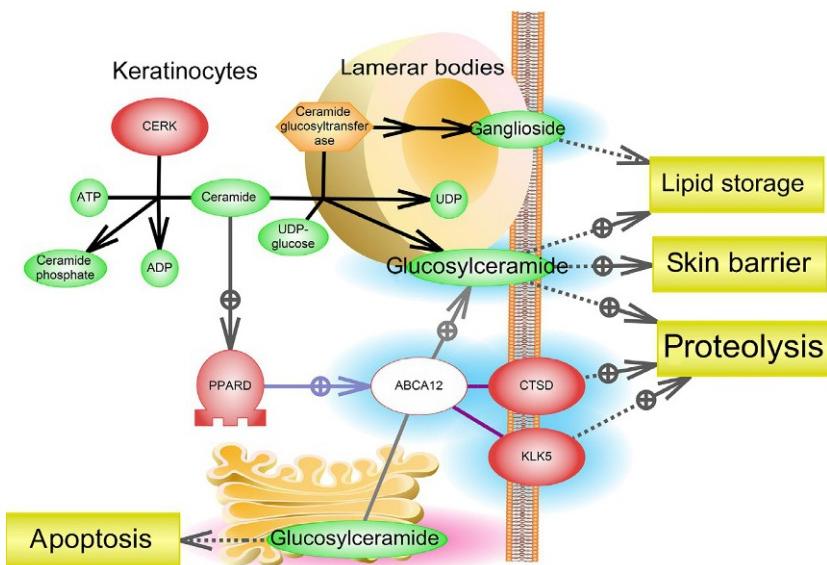
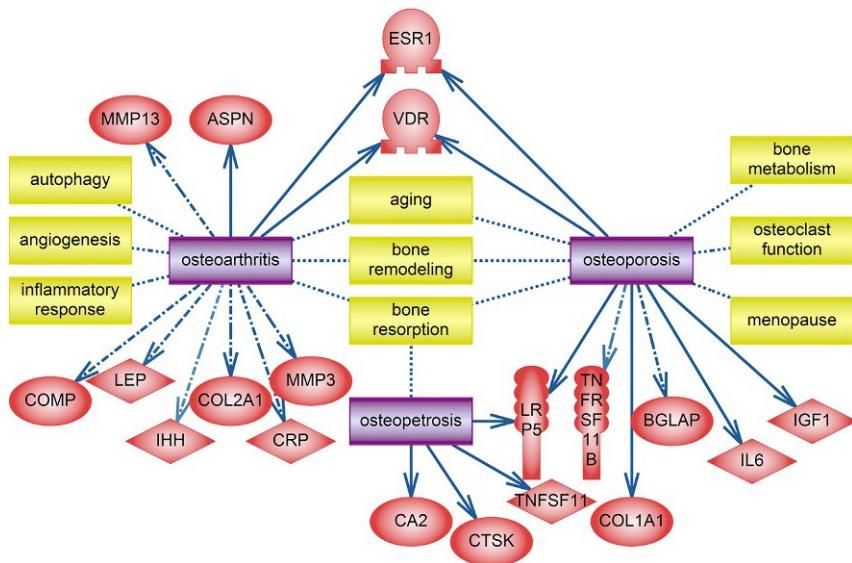


FIG. 10 Pathway 3: Harlequin ichthyosis overview.

## References

- Disease number #308100, #146700, #607800 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code Q80. Diseases of the skin and subcutaneous tissue (L00-L99). (ICD-10, <https://icdlist.com>). ICD-11: disease code EC20.0.
- Akiyama, M., 2014. The roles of ABCA12 in epidermal lipid barrier formation and keratinocyte differentiation. *Biochim. Biophys. Acta* 1841, 435–440. <https://doi.org/10.1016/j.bbapap.2013.08.009>.
- Elias, P.M., Williams, M.L., Choi, E.-H., Feingold, K.R., 2014. Role of cholesterol sulfate in epidermal structure and function: lessons from X-linked ichthyosis. *Biochim. Biophys. Acta* 1841, 353–361. <https://doi.org/10.1016/j.bbapap.2013.11.009>.
- Hernández-Martín, A., González-Sarmiento, R., De Unamuno, P., 1999. X-linked ichthyosis: an update. *Br. J. Dermatol.* 141, 617–627. <https://doi.org/10.1046/j.1365-2133.1999.03098.x>.
- Hernández-Martin, A., Aranegui, B., Martin-Santiago, A., Garcia-Doval, I., 2013. A systematic review of clinical trials of treatments for the congenital ichthyoses, excluding ichthyosis vulgaris. *J. Am. Acad. Dermatol.* 69, 544–549. e8. <https://doi.org/10.1016/j.jaad.2013.05.017>.
- Kezic, S., Jakasa, I., 2016. Filaggrin and skin barrier function. *Curr. Probl. Dermatol.* 49, 1–7. <https://doi.org/10.1159/000441539>.
- Kitajima, Y., 2015. Implications of normal and disordered remodeling dynamics of corneodesmosomes in stratum corneum. *Dermatol. Sin.* 33, 58–63. <https://doi.org/10.1016/j.dsi.2015.03.009>.
- Marukian, N.V., Choate, K.A., 2016. Recent advances in understanding ichthyosis pathogenesis. *F1000Research* 5, <https://doi.org/10.12688/f1000research.8584.1>.
- Rodríguez-Pazos, L., Ginarte, M., Vega, A., Toribio, J., 2013. Autosomal recessive congenital ichthyosis. *Actas Dermosifiliogr.* 104, 270–284. <https://doi.org/10.1016/j.adengl.2011.11.021>.
- Takeichi, T., Akiyama, M., 2016. Inherited ichthyosis: non-syndromic forms. *J. Dermatol.* 43, 242–251. <https://doi.org/10.1111/1346-8138.13243>.
- Thyssen, J.P., Godoy-Gijón, E., Elias, P.M., 2013. Ichthyosis vulgaris: the filaggrin mutation disease. *Br. J. Dermatol.* 168, 1155–1166. <https://doi.org/10.1111/bjd.12219>.
- Uchida, Y., 2014. Ceramide signaling in mammalian epidermis. *Biochim. Biophys. Acta* 1841, 453–462. <https://doi.org/10.1016/j.bbapap.2013.09.003>.
- Wu, B., Paller, A.S., 2017. Ichthyosis, X-linked. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Zuo, Y., Zhuang, D.Z., Han, R., Isaac, G., Tobin, J.J., McKee, M., Welti, R., Brissette, J.L., Fitzgerald, M.L., Freeman, M.W., 2008. ABCA12 maintains the epidermal lipid permeability barrier by facilitating formation of ceramide linoleic esters. *J. Biol. Chem.* 283, 36624–36635. <https://doi.org/10.1074/jbc.M807377200>.

# Diseases of the musculoskeletal system



## OUTLINE

Osteoarthritis	535
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Osteopetrosis	557

There is a broad spectrum of musculoskeletal disorders. They can be classified as arthritis (osteoarthritis and other types), connective tissue disorders (rheumatoid arthritis, systemic lupus erythematosus, and others), skeletal muscle diseases (polymyositis and others), bone diseases (osteopetrosis, osteoporosis, and others), and musculoskeletal neoplasias.

Pathologies of skeletal muscle and connective tissue are usually closely related to the immune and nervous systems. Together, they involve cell differentiation pathways, and therefore they deserve several separate chapters. This chapter focuses on selected bone diseases.

Osteoarthritis and osteoporosis are common bone diseases among the elderly.

Osteoarthritis is the most common form of arthritis that causes joint pain, stiffness, and results in a breakdown of joint cartilage and underlying bone.

Osteoporosis is the most common cause of bone fractures among the elderly, especially in women because of decreased bone density after menopause that results from lower levels of estrogen expression.

On the contrary, osteopetrosis makes bones abnormally dense and prone to fracture. Osteopetrosis is a rare genetic disorder affecting about 1 in 20,000 people. Nevertheless, it is a worthy example of a complex syndrome-like disease, which can be caused by mutations in several genes, and therefore it illustrates the multifactorial nature of bone-related diseases.

## CHAPTER

## 12.1

## Osteoarthritis

Osteoarthritis is a common disease of the joints that primarily occurs in older adults. This condition is characterized by the breakdown of cartilage, the tough but flexible tissue that covers the ends of the bones at the joints and allows smooth joint movements. One or more parts of the body can be affected, most often the hands, shoulders, spine, knees, or hips (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Osteoarthritis (OA) is a progressive disease of the joint representing failed repair of joint damage. (*Ferri and Ferri, 2018*).

Abnormalities may initiate intraarticular stresses that lead to joint damage in articular cartilage, subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves, or the synovium. The result is the breakdown of cartilage and bone leading to symptoms of pain, stiffness, and functional disability (*Ferri and Ferri, 2018*).

Approximately 80% of the population over the age of 65 years displays radiographic evidence of OA. Treatment of OA is limited to the use of pain-killer medications such as nonsteroidal antiinflammatory drugs (NSAIDs) and joint replacement surgery (*Charlier et al., 2016*).

The etiology of OA is not well known. The risk factors for primary OA include a combination of heredity and systemic health changes (e.g., obesity and age). Secondary OA (posttraumatic arthritis) develops after accidental or chronic joint trauma. Hereditarily of osteoarthritis is complex and poorly understood. Currently, more than 50 genes or gene loci have been associated with OA (*Wang et al., 2016*).

The excessive mechanical loading of cartilage is a proven trigger of osteoarthritis. The mechanical stress initiates the release of proinflammatory cytokines, which alter the normal function of chondrocytes and lead to cartilage damage:

**Pathway 1.** *TNF and IL1B provoke ECM degradation in osteoarthritis* (**Fig. 1**). Inflammation, mechanical, and oxidative stress and other factors induce the apoptosis of chondrocytes:

**Pathway 2.** *Chondrocyte death in osteoarthritis* (**Fig. 2**).

Cartilage ossification and chondrocyte hypertrophy characteristic of osteoarthritis are its other main pathogenic features although their cause and underlying mechanisms are still not fully understood:

**Pathway 3.** *TGFB signaling provokes endochondral ossification with osteophyte formation in OA (Fig. 3).*

## Key cellular contributors and processes

### Apoptosis

#### Process

Apoptosis is a highly regulated chain of events leading to cell destruction that occurs in multicellular organisms. Apoptosis eliminates damaged or redundant cells and is required for normal tissue development and homeostasis.

### Autophagy

#### Process

Autophagy is a conserved eukaryotic process in which excessive or dysfunctional intracellular components are delivered to lysosomes for degradation. The three major types of autophagy include macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membrane vesicle, the autophagosome.

### Chondrocyte

#### Cell

Chondrocytes are the only cell type present in healthy cartilage tissue. Chondrocytes are responsible for the synthesis and turnover of the cartilaginous ECM, whose main components are collagens and proteoglycans.

### Chondroptosis

#### Process

The term chondroptosis refers to the process of nontypical programmed death (apoptosis) in chondrocytes with specific features such as cytoplasmic vacuolization without nuclear fragmentation. Chondroptosis is a highly regulated process required for cartilage degradation during skeleton development.

### Endochondral ossification

#### Process

Endochondral ossification is the process of bone development in which growing cartilage is gradually replaced by bone tissue.

### Necrosis

#### Process

Necrosis is a premature death of living cells by autolysis caused by disease, trauma, or insufficient blood supply to the organ or tissue.

## Pathway 1

### TNF and IL1B provoke ECM degradation in osteoarthritis (Fig. 1)

#### Incoming signals

The excessive mechanical loading of cartilage causes not only direct damage of the tissue but also increased proinflammatory cytokines synthesis by activated synoviocytes, mononuclear cells, and cartilage itself (Fernandes et al., 2002). Proinflammatory cytokines switch the catabolic activity of chondrocytes to produce higher levels of extracellular matrix (ECM) degrading enzymes. Adipose tissue also may release proinflammatory cytokines and adipokines (not shown in the pathway), which participate in the degradation of cartilage in OA. That, along with excessive mechanical loading, is why obesity is one of the risk factors for OA.

#### Outcome effects

The cartilage ECM is made of water, collagens (type II), proteoglycans, and hydrophilic macromolecules. Overexpressed in OA, aggrecanases, metalloproteinases, and collagenases break down components of the cartilage ECM. The progressive inflammation in OA leads to cartilage degradation and a breakdown of all joint tissues (bone, synovium, and ligaments) along with the appearance of new bone outgrowth at the joint margins (osteophytes), synovial inflammation, and sclerosis of subchondral bone (Dreier, 2010; Lotz, 2012; Wang et al., 2011).

#### Signaling

Proinflammatory cytokines such as TNF, IL1B, and IL6 activate NF- $\kappa$ B, the toll-like receptor (TLR), ERK/MAPK, and other signaling pathways in chondrocytes to promote the overexpression of enzymes that cleave ECM in cartilage (Charlier et al., 2016). Only the major TNF and IL1B receptor signalings are shown on the pathway. Proinflammatory cytokines induce the overexpression of matrix metalloproteinases and collagenases (MMP1/3/8/13) and aggrecanases (e.g., ADAMTS4/ADAMTS5) in chondrocytes starting from the early stages of OA. The degradation of a member of the aggrecan/versican proteoglycan family in cartilage tissue, ACAN, impairs the resistance of cartilage to mechanical stress.

Chondrocytes also produce nitric oxide (NO), which has both protective and proapoptotic effects on cartilage. The synthesis of prostaglandin E2

by PTGES and PTGS2 provokes pain (arthralgia). During inflammation, chondrocytes synthesize high levels of type X collagen (COL10A1), which in turn stimulates bone tissue formation (endochondral ossification) in articular cartilage ([Shen, 2005](#)). Synthesis of excess levels of VGFA by chondrocytes stimulates neovascularization of avascular cartilage tissue to promote endochondral ossification ([Murata et al., 2008](#)).

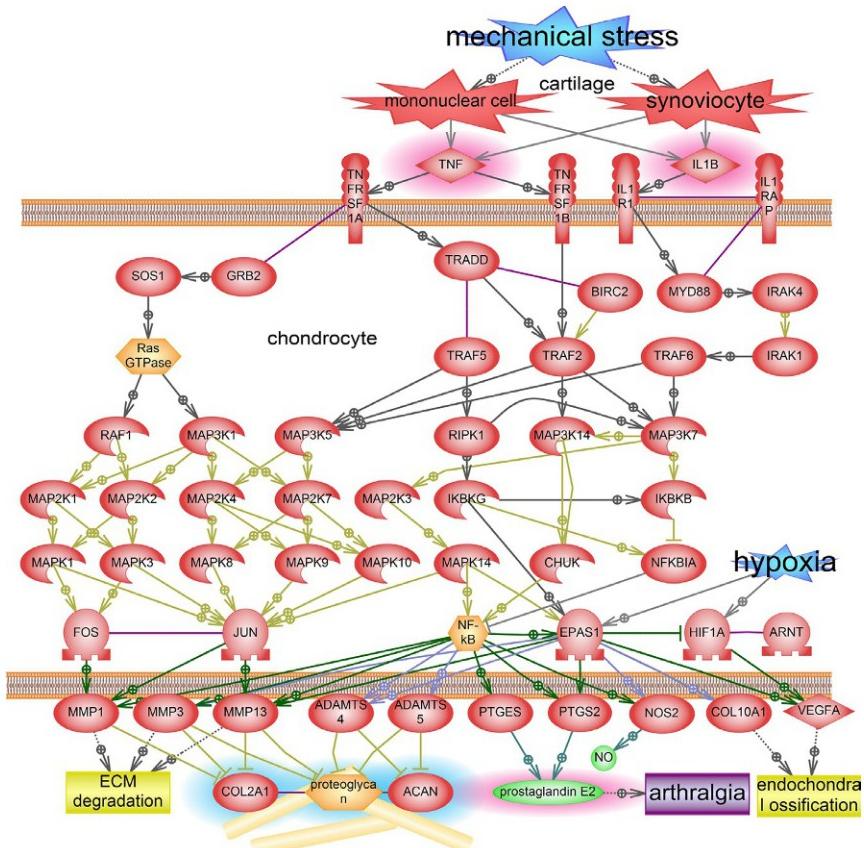


FIG. 1 Pathway 1: TNF and IL1B provoke ECM degradation in osteoarthritis.

## Pathway 2

### Chondrocyte death in osteoarthritis (Fig. 2)

#### Incoming signals

Cartilage and ECM damage in OA is strongly associated with chondrocyte death although it is unclear what comes first. Chondrocytes die through a combination of apoptosis, necrosis, autophagy, and chondroptosis. Proinflammatory cytokines may promote chondrocyte death together ECM damage, oxidative, and mechanical stress.

#### Outcome effects

Cartilage tissue is composed of ECM and only one cell type, the chondrocytes that synthesize the matrix. Adult chondrocytes seldom divide and therefore display almost no cellular turnover. If chondrocytes die or become dysfunctional, maintenance of the proper supportive and lubricative functions of cartilage becomes difficult to restore, especially given the fact that the ECM is neither innervated and nor vascularized.

#### Signaling

TNF promotes chondrocyte death through two mechanisms involving the direct induction of apoptosis and the indirect priming of cells for apoptosis by FAS/FASL. Apoptosis stimulants include activation of the caspases cascade with the help of mitochondrial proteins (BID, BAX, and CYCS) as well as MAPKs signaling.

IL1B promotes effects similar to the role of TNF cascades that initiate cell death. Also, IL1B supports the effects of numerous miRNAs in the chondrocyte life cycle (not shown).

The initiation of autophagy may avoid chondrocyte death in the early stages of OA. Although robust autophagy, later on, may also lead to cell death ([Charlier et al., 2016](#)). The hypoxia-inducible heterodimer HIF1a/HIF1b (ARNT) present under normal avascular cartilage hypoxic conditions becomes an activated transcription factor, which maintains the chondrocyte phenotype. HIF1a is likely to promote the shift from apoptosis to autophagy (through mitochondrial BCL2 AMPK/mTOR signaling) in chondrocytes. HIF2 (EPAS1) is an inhibitor of autophagy, and it stimulates both cartilage destruction and chondrocyte cell death. Expression level of EPAS1 in cartilage is high in the earlier stage of OA and low at later stages ([Charlier et al., 2016](#)).

Necrotic cell death (necrosis) occurs under conditions of extreme tissue damage, such as ischemia or trauma, when apoptosis fails to initiate.

The mechanical damage of membranes leads to the release of free radicals of hydrogen, oxygen, and other elements. Free radicals lead to lipid peroxidation and the destruction of cellular membranes. Oxidative stress directly leads to the damage of cellular macromolecules including DNA, proteins, and lipids. Programmed necrosis (necroptosis) is a regulated necrotic cell death pathway controlled by TNF signaling and the RIPK1/RIPK3 kinases complex in OA ([Lotz, 2012](#); [Saito and Tanaka, 2017](#); [Vincenti and Brinckerhoff, 2002](#)).

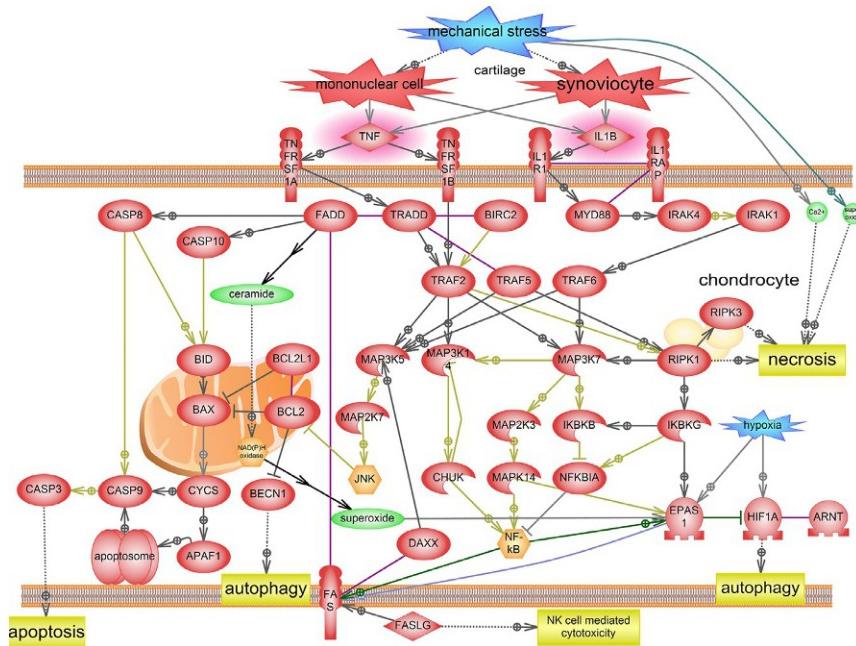


FIG. 2 Pathway 2: Chondrocyte death in osteoarthritis.

## Pathway 3

### TGFB signaling provokes endochondral ossification with osteophyte formation in OA ([Fig. 3](#))

#### Incoming signals

Alterations of the mechanisms leading to the terminal differentiation of chondrocytes could be a major reason for OA development. Chondrocytes differentiate only during childhood and early adolescence in the growth plate of long bones.

Nevertheless, changes of the adult chondrocyte phenotype in OA have characteristics similar to chondrogenesis (such as hypertrophic enlargement and expression of COL10A1, MMP13, and BGLAP) and may be associated with the process of endochondral ossification of the cartilage in this disease. Normally, endochondral ossification is essential for the growth and repair of bones. However, OA is thought to be stimulated by enhanced bone mass formation and protected by osteoporosis ([van der Kraan et al., 2012](#)). Details of this process in human cartilage have yet to be revealed.

TGFB signaling is the primary stimulator of terminal chondrocyte differentiation.

Mutations in genes that encode proteins involved with the TGFB-related cascades have strong associations with OA development in some populations. Those genes include *TGF $\beta$ 1* (rs2227306), *SMAD3* (rs12901499), and *GDF5* (rs143383). Polymorphisms in other genes associated with OA, such as *DVWA* (also known as collagen type VI alpha 4 pseudogene 1, *COL6A4P1*), also may play a role in chondrocyte differentiation ([Wang et al., 2016](#)).

#### Outcome effects

Overexpression of MMP13, COL10A1, BGLAP, SPP1, and SPARC by chondrocytes in patients with OA provokes endochondral ossification and disease progression ([Lotz, 2012](#); [van der Kraan and van den Berg, 2012](#); [Wang et al., 2011](#); [Xia et al., 2014](#); [Yang et al., 2001](#)).

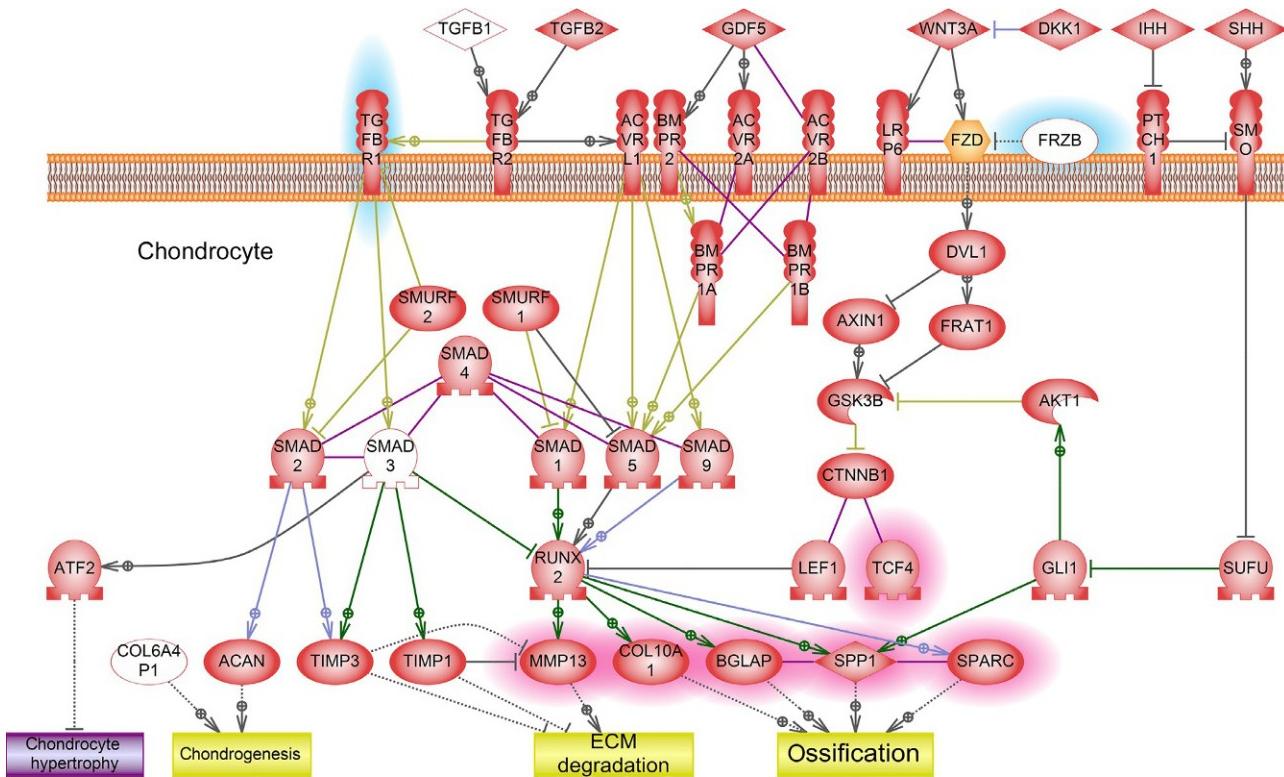
#### Signaling

The TGFBs play a dual role in chondrocyte function. Acting through the TGFBR1 receptor and the SMAD2/SMAD3 complex they inhibit chondrocyte terminal differentiation and chondrocyte hypertrophy. When TGFBR1 signaling is inhibited, the ACVRL1 receptor and the SMAD1/SMAD5/SMAD8 complex are activated, leading to chondrocyte terminal

differentiation. For example, TGFB signaling relieves MMP13 from the pressure imposed by tissue inhibitors of metalloproteinase (TIMPs). RUNX2 is the primary downstream nuclear transcription factor in chondrogenesis. RUNX2 controls the expression of the MMP13, COL10A1, BGLAP, SPP1, and SPARC proteins, which maintain the hypertrophic characteristic of chondrocytes.

The WNT, BMP, and SHH signaling pathways, among others, regulate SMAD complexes and determine cell fate during chondrogenesis. For example, some laboratories reported elevated levels of TCF4 expression (one of the markers of WNT activity) in human OA cartilage compared with healthy cartilage. Others report a loss of function polymorphism in *FRZB* (an inhibitor of WNT signaling) that is associated with female-specific OA. However, interactions of different signaling pathways in human chondrocytes derived from patients with OA require further study, because to date, researchers have obtained conflicting experimental data (Ma et al., 2013; Wang et al., 2016).

## II. Human disease pathways



**FIG. 3** Pathway 3: TGFB signaling provokes endochondral ossification with osteophyte formation in OA.

## References

- Disease number # 165720, # 612400 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code M15/M19/M47. Diseases of the musculoskeletal system and connective tissue (M00-M99). (ICD-10, <https://icdlist.com>). ICD-11: disease code FA0Z/FA05.
- Charlier, E., Relic, B., Deroyer, C., Malaise, O., Neuville, S., Collée, J., Malaise, M.G., De Seny, D., 2016. Insights on molecular mechanisms of chondrocytes death in osteoarthritis. *Int. J. Mol. Sci.* 17. <https://doi.org/10.3390/ijms17122146>.
- Dreier, R., 2010. Hypertrophic differentiation of chondrocytes in osteoarthritis: the developmental aspect of degenerative joint disorders. *Arthritis Res. Ther.* 12, 216. <https://doi.org/10.1186/ar3117>.
- Fernandes, J.C., Martel-Pelletier, J., Pelletier, J.-P., 2002. The role of cytokines in osteoarthritis pathophysiology. *Biorheology* 39, 237–246.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Lotz, M., 2012. Osteoarthritis year 2011 in review: biology. *Osteoarthr. Cartil.* 20, 192–196. <https://doi.org/10.1016/j.joca.2011.11.015>.
- Ma, B., Zhong, L., van Blitterswijk, C.A., Post, J.N., Karperien, M., 2013. T cell factor 4 is a pro-catabolic and apoptotic factor in human articular chondrocytes by potentiating nuclear factor κB signaling. *J. Biol. Chem.* 288, 17552–17558. <https://doi.org/10.1074/jbc.M113.453985>.
- Murata, M., Yudoh, K., Masuko, K., 2008. The potential role of vascular endothelial growth factor (VEGF) in cartilage: how the angiogenic factor could be involved in the pathogenesis of osteoarthritis? *Osteoarthr. Cartil.* 16, 279–286. <https://doi.org/10.1016/j.joca.2007.09.003>.
- Saito, T., Tanaka, S., 2017. Molecular mechanisms underlying osteoarthritis development: notch and NF-κB. *Arthritis Res. Ther.* 19, 94. <https://doi.org/10.1186/s13075-017-1296-y>.
- Shen, G., 2005. The role of type X collagen in facilitating and regulating endochondral ossification of articular cartilage. *Orthod. Craniofac. Res.* 8, 11–17. <https://doi.org/10.1111/j.1601-6343.2004.00308.x>.
- van der Kraan, P.M., van den Berg, W.B., 2012. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? *Osteoarthr. Cartil.* 20, 223–232. <https://doi.org/10.1016/j.joca.2011.12.003>.
- van der Kraan, P.M., Goumans, M.-J., Blaney Davidson, E., ten Dijke, P., 2012. Age-dependent alteration of TGF-β signalling in osteoarthritis. *Cell Tissue Res.* 347, 257–265. <https://doi.org/10.1007/s00441-011-1194-6>.
- Vincenti, M.P., Brinckerhoff, C.E., 2002. Transcriptional regulation of collagenase (MMP-1, MMP-13) genes in arthritis: integration of complex signaling pathways for the recruitment of gene-specific transcription factors. *Arthritis Res.* 4, 157–164. <https://doi.org/10.1186/ar401>.
- Wang, M., Shen, J., Jin, H., Im, H.-J., Sandy, J., Chen, D., 2011. Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. *Ann. N. Y. Acad. Sci.* 1240, 61–69. <https://doi.org/10.1111/j.1749-6632.2011.06258.x>.
- Wang, T., Liang, Y., Li, H., Li, H., He, Q., Xue, Y., Shen, C., Zhang, C., Xiang, J., Ding, J., Qiao, L., Zheng, Q., 2016. Single nucleotide polymorphisms and osteoarthritis: an overview and a meta-analysis. *Medicine (Baltimore)* 95, e2811. <https://doi.org/10.1097/MD.0000000000002811>.
- Xia, B., Chen, D., Zhang, J., Hu, S., Jin, H., Tong, P., 2014. Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif. Tissue Int.* 95, 495–505. <https://doi.org/10.1007/s00223-014-9917-9>.
- Yang, X., Chen, L., Xu, X., Li, C., Huang, C., Deng, C.X., 2001. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J. Cell Biol.* 153, 35–46.

## CHAPTER

## 12.2

Osteoporosis (type I)

Osteoporosis is caused by a shortage of calcium and other minerals in bones (decreased bone mineral density), which makes the bones brittle and prone to fracture. Osteoporosis occurs when the new bone tissue remodeling delays toward the removal of old bone tissues (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Osteoporosis is characterized by increased bone fragility and a higher fracture risk caused by a progressive decrease in bone mass. (*Ferri and Ferri, 2018*).

Two forms of osteoporosis are known. Primary osteoporosis affects 80% of women and 60% of men and includes idiopathic osteoporosis, type I osteoporosis (estrogen related) and type II osteoporosis. Idiopathic osteoporosis is a disease for which the mechanisms of pathogenesis are unknown and which may occur in children and young adults. Type I osteoporosis occurs in postmenopausal women (ages 51–75), and it is characterized by accelerated trabecular bone loss, and it is associated with the vertebral body and distal forearm fractures (estrogen withdrawal effect). Type II osteoporosis (involutional) occurs in both men and women over 70 years old. Type II osteoporosis is characterized by both trabecular and cortical bone loss, and it is associated with fractures of the proximal humerus and tibia, femoral neck, and pelvis.

Secondary osteoporosis affects 20% of women and 40% of men. It exists as a common feature of another disease process, a heritable disorder of connective tissue or as a treatment side effect (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Here, only type I osteoporosis (estrogen related) is described. Other types of osteoporosis either lack sufficient research findings or have complex mechanisms involving cell redifferentiation pathways.

A lack of estrogens causes the activation of osteoclasts, which leads to bone resorption in type I osteoporosis:

**Pathway 1.** *Osteoclast-mediated bone resorption in postmenopausal women (Fig. 4).*

In postmenopausal osteoporosis, a deficiency of estrogens may provoke osteoblast dysfunction. The low rate of osteoblast survival and differentiation leads to reduced bone tissue formation:

**Pathway 2.** *Osteoblast-mediated bone demineralization in postmenopausal women (Fig. 5).*

## Key cellular contributors and processes

### Osteoblast

#### Cell

An osteoblast is a specialized connective tissue-related bone cell responsible for the synthesis and mineralization of bone during the initial bone formation, as well as bone remodeling.

### Osteoclast

#### Cell

Osteoclasts are giant multinuclear bone cells of hematopoietic origin responsible for dissolution and absorption of bone. This function is critical for the maintenance, repair, and remodeling of bones in the vertebral skeleton.

## Pathway 1

### Osteoclast-mediated bone resorption in postmenopausal women [\(Fig. 4\)](#)

#### Incoming signals

The osteoclast is a bone cell that breaks down bone tissue. This process is critical to the maintenance and repair of healthy bones. Bone surfaces undergo demineralization due to increased proton secretion by activated osteoclasts. An estrogen deficiency may induce osteoclast activation. Usually, estrogens prevent bone loss by blocking the production of proinflammatory cytokines such as TNF by bone marrow and bone cells and by preventing excess osteoclast differentiation ([Inada and Miyaura, 2010; Riggs, 2000](#)).

#### Outcome effects

Osteoclast-mediated bone resorption can be divided into two steps: demineralization by the secretion of H<sup>+</sup> ions (i.e., protons) onto the bone surface and the subsequent proteolytic resorption of exposed organic matrix primarily composed primarily of collagen type I. The degradation of bone matrix and demineralization causes the bone loss characteristic of osteoporosis.

#### Signaling

Estrogen suppresses production of the soluble receptor tumor necrosis factor receptor superfamily, member 11b (TNFRSF11B) by peripheral blood mononuclear cells ([Rachner et al., 2008](#)) (the signaling is not shown). In postmenopausal women, the deficiency of estrogens leads to insufficient TNFRSF11B expression and an increase in the TNF superfamily member 11 (TNFSF11) to TNFRSF11B ratio. TNFSF11 (also known as RANKL) initiates osteoclast differentiation by binding to the TNFRSF11A receptor on the osteoclast surface. Also, estrogen may suppress tumor necrosis factor (TNF) production in bone ([Vural et al., 2006](#)). TNF affects the same NF- $\kappa$ B and MAPK pathways as TNFSF11 through the activation of TNF receptor superfamily members 1A/B (TNFRSF1A and TNFRSF1B) receptor signaling. In addition, TNFSF11 promotes osteoclast survival through the AKT1-dependent pathway.

The NF- $\kappa$ B and MAPK pathways eventually trigger nuclear factor of activated T-cell (NFATC1)-mediated cathepsin K (CTSK) and matrix metallopeptidase 13 (MMP13) expression in osteoclasts, which can destroy the bone directly. NFATC1 also controls expression of the calcitonin receptor (CALCR), which maintains calcium homeostasis in bone. Polymorphisms

in the *CALCR* gene correlated with lower bone mineral density in postmenopausal women (OMIM: #114131, [www.omim.org](http://www.omim.org)).

Osteoclast-mediated bone resorption occurs via ruffled border membranes induced by protons secreted from osteoclasts. Protons are formed within osteoclasts as a result of the dissociation of carbonic acid through the action of carbonic anhydrase 2 (CA2) from CO<sub>2</sub> and H<sub>2</sub>O, and they are pumped out of the cells by V-ATPase. Protons on the bone surface provide the low pH required for the activation and function of CTSK, and it also promotes the release of Ca<sup>2+</sup> from hydroxylapatite. CTSK degrades collagen type I in the telopeptide regions and cleaves collagen triple helical domains at multiple sites (Raisz, 2005; Yasuda et al., 2005).

## II. Human disease pathways

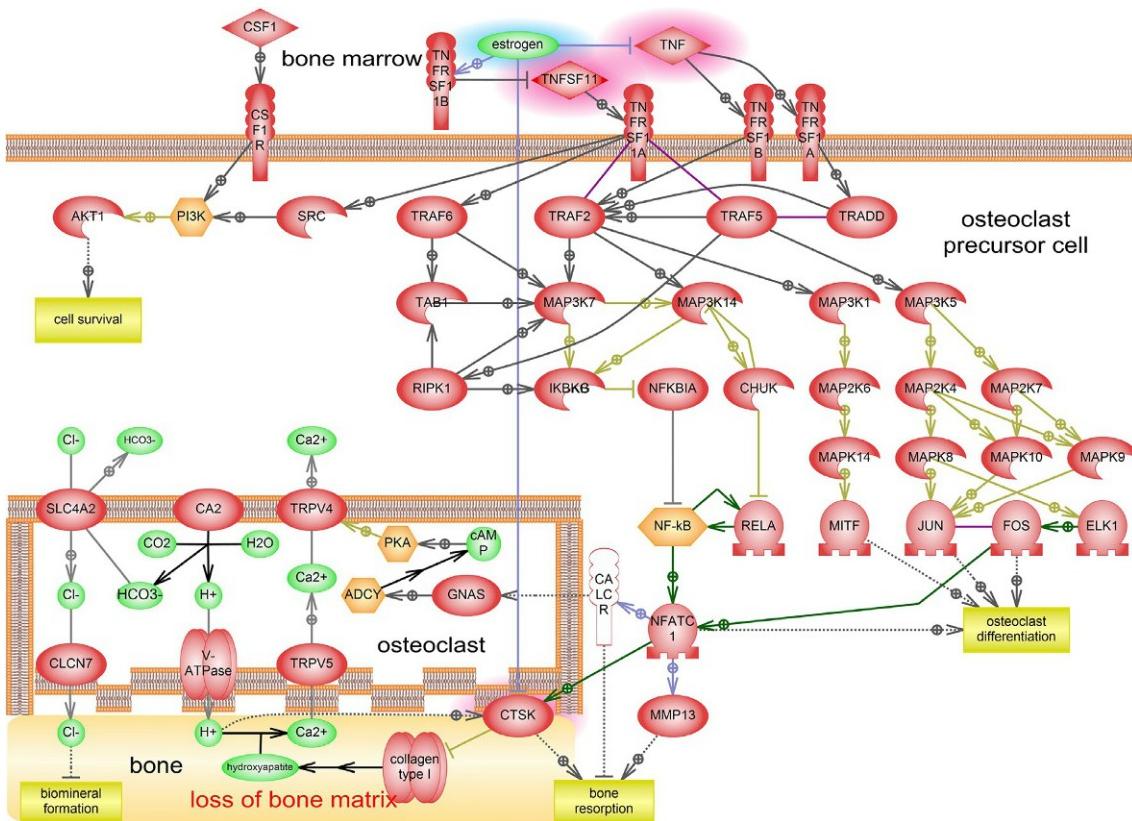


FIG. 4 Pathway 1: Osteoclast-mediated bone resorption in postmenopausal women.

## Pathway 2

### Osteoblast-mediated bone demineralization in postmenopausal women (Fig. 5)

#### Incoming signals

Osteoblasts are the bone cells responsible for the synthesis and mineralization of bone tissue during both embryonic bone formation and later bone remodeling. Collagen type I and hydroxylapatite are key components of the healthy bone matrix. Hydroxylapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ ) is formed in membrane-bound matrix vesicles (MV) within osteoblasts. Usually, estrogen acts via the estrogen receptor 1 (ESR1) resulting in osteoblast proliferation and differentiation. In postmenopausal osteoporosis, the lack of osteoblast function is due to a deficiency of estrogens.

#### Outcome effects

The deficiency of estrogens and polymorphisms in the genes encoding osteoclast-specific proteins leads to impaired osteoblast function affecting bone matrix formation and bone biomineralization (Lodewyckx and Lories, 2009; Raisz, 2005).

#### Signaling

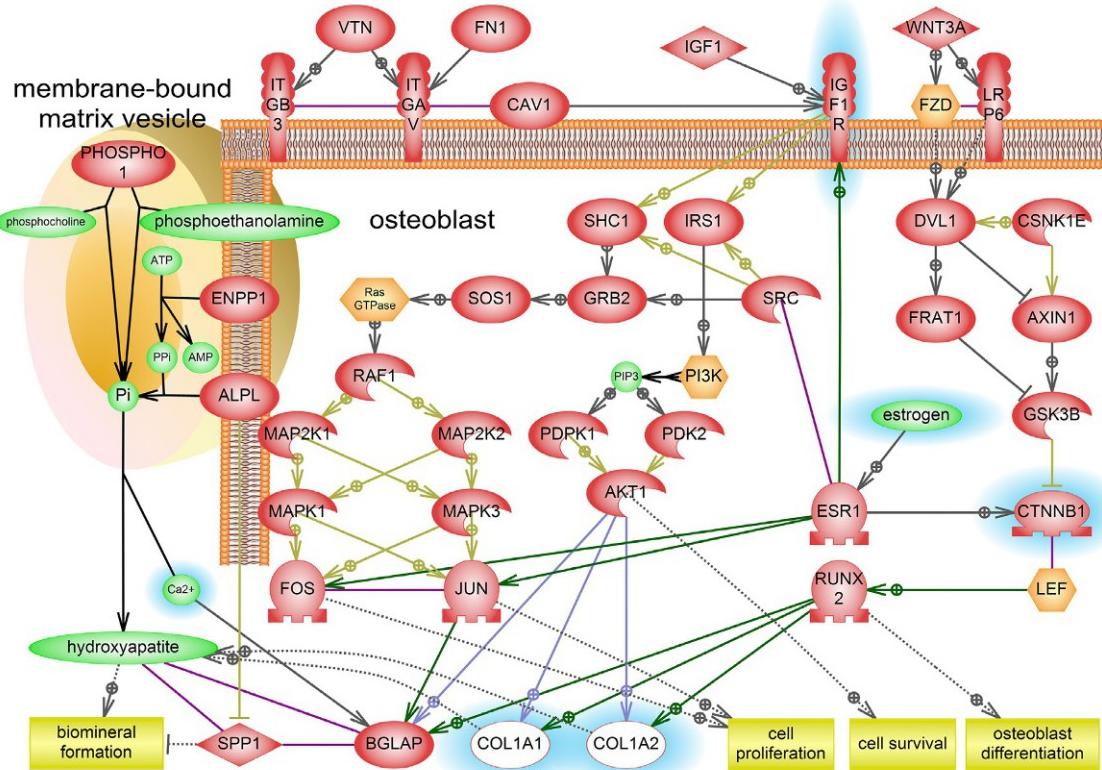
Estrogen promotes both genomic and nongenomic ESR1-mediated intracellular signaling. ESR1 activates the expression of insulin-like growth factor 1 receptor (IGF1R) and stimulates the WNT/CTNNB1 and other cascades. The IGF1R signaling includes downstream activation of the AKT1 and MAPK pathway leading to cellular proliferation and increased cell survival. The canonical WNT pathway is responsible for osteoblast differentiation involving the runt-related transcription factor 2 (RUNX2) transcription factor.

The differentiated and functional osteoclast is vital for normal calcium utilization and hydroxylapatite formation. These processes include several steps. Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is a membrane protein of MV, which converts ATP into AMP with the release of PPi. PPi is then hydrolyzed by the alkaline phosphatase (ALPL) enzyme located in the MV outer membrane, yielding monophosphate ions (Pi) that are then incorporated into a bone mineral. Also, another protein, phosphoethanolamine/phosphocholine phosphatase (PHOSPHO1), hydrolyzes phosphorylcholine and phosphoethanolamine to produce Pi inside the MV. Pi complexes with  $\text{Ca}^{2+}$  in the form of hydroxylapatite crystal. The osteoclasts' MV initially deposits hydroxylapatite as noncrystalline compounds

that are subsequently converted into hydroxylapatite, the main crystalline salt of bone. Hydroxylapatite stores ions and gives bone its compressional strength. Some hydroxylapatite remains in the noncrystalline form to facilitate a rapid bone tissue resorption.

Osteoblasts synthesize proteins, which compose the organic matrix of bone including collagen or the bone gamma-carboxyglutamate protein (BGLAP). Mutations in genes encoding bone matrix proteins may change the formation of cross-linking of collagen, which challenges the process of normal mineral and matrix composition. Some variations in the collagen type I alpha 1 chain/alpha 2 chain (*COL1A1* and *COL1A2*) genes cause a dominant monogenetic osteoporotic disease, osteogenesis imperfecta (OMIM: # 120150, [www.omim.org](http://www.omim.org)) (Majchrzycki et al., 2015; Raisz, 2005; Ralston and Uitterlinden, 2010; Zofkova et al., 2015).

## II. Human disease pathways



**FIG. 5** Pathway 2: Osteoblast-mediated bone demineralization in postmenopausal women.

## References

- Disease number # 166710 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code M80—M82. Diseases of the musculoskeletal system and connective tissue (M00-M99). (ICD-10, <https://icdlist.com>). ICD-11: disease code FB83.1.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Inada, M., Miyaura, C., 2010. Cytokines in bone diseases. Cytokine and postmenopausal osteoporosis. Clin. Calcium 20, 1467–1472. <https://doi.org/CliCa101014671472>.
- Lodewyckx, L., Lories, R.J.U., 2009. WNT signaling in osteoarthritis and osteoporosis: what is the biological significance for the clinician? Curr. Rheumatol. Rep. 11, 23–30.
- Majchrzycki, M., Bartkowiak-Wieczorek, J., Wolski, H., Drews, K., Bogacz, A., Czerny, B., Zagrodnik-Ulan, E., Seremak-Mrozikiewicz, A., 2015. Polymorphisms of collagen 1A1 (COL1A1) gene and their relation to bone mineral density in postmenopausal women. Ginekol. Pol. 86, 907–914.
- Rachner, T.D., Schoppet, M., Niebergall, U., Hofbauer, L.C., 2008. 17beta-Estradiol inhibits osteoprotegerin production by the estrogen receptor-alpha-positive human breast cancer cell line MCF-7. Biochem. Biophys. Res. Commun. 368, 736–741. <https://doi.org/10.1016/j.bbrc.2008.01.118>.
- Raisz, L.G., 2005. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J. Clin. Invest. 115, 3318–3325. <https://doi.org/10.1172/JCI27071>.
- Ralston, S.H., Uitterlinden, A.G., 2010. Genetics of osteoporosis. Endocr. Rev. 31, 629–662. <https://doi.org/10.1210/er.2009-0044>.
- Riggs, B.L., 2000. The mechanisms of estrogen regulation of bone resorption. J. Clin. Invest. 106, 1203–1204. <https://doi.org/10.1172/JCI11468>.
- Vural, P., Akgul, C., Canbaz, M., 2006. Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. Pharmacol. Res. 54, 298–302. <https://doi.org/10.1016/j.phrs.2006.06.006>.
- Yasuda, Y., Kaleta, J., Brömme, D., 2005. The role of cathepsins in osteoporosis and arthritis: rationale for the design of new therapeutics. Adv. Drug Deliv. Rev. 57, 973–993. <https://doi.org/10.1016/j.addr.2004.12.013>.
- Zofkova, I., Nemcikova, P., Kuklik, M., 2015. Polymorphisms associated with low bone mass and high risk of atraumatic fracture. Physiol. Res. 64, 621–631.

## CHAPTER

## 12.3

## Osteopetrosis

Osteopetrosis (known as marble bone disease) is a group of rare heritable disorders of the skeleton characterized by increased bone density visible on radiographs. Osteopetrotic conditions vary in their presentation and severity, ranging from neonatal onset with life-threatening complications such as bone marrow failure to the incidental finding of osteopetrosis on radiographs. Moreover, individuals with osteopetrosis may develop a form of kidney disease called renal tubular acidosis.

Osteopetrosis is a bone disease that makes bones abnormally dense and prone to breakage (fracture). Researchers have described several major types of osteopetrosis, which are usually distinguished by their pattern of inheritance: autosomal dominant, autosomal recessive, or X-linked. (*Genetics Home Reference*, <https://ghr.nlm.nih.gov>).

Failure of osteoclast development and function is the leading cause of osteopetrosis. Mutations in at least 10 genes have been associated with osteopetrosis.

The leading causes of osteoclast dysfunction are multiple mutations in the genes involved in osteoclast function:

**Pathway 1.** *Osteoclast functional impairment in osteopetrosis (Fig. 6).*

Insufficient production of TNFSF11 by osteoblasts can result in osteoclast dysfunction:

**Pathway 2.** *WNT signaling dysregulation in osteoblasts (Fig. 7).*

A rare form of osteopetrosis is associated with renal disorder:

**Pathway 3.** *Renal tubular acidosis associated with carbonic anhydrase II mutation and osteopetrosis (Fig. 8).*

## Key cellular contributors and processes

Bone remodeling

Process

Bone remodeling is a dynamic process, which maintains bone strength and ion homeostasis by replacing discrete parts of old bone with newly synthesized bone matrix. While bone resorption is performed by large immune cells called osteoclasts, osteoblasts, a type of specialized connective tissue-related cells, is responsible for making new bone. Bone remodeling is impaired in osteopetrosis due to inadequate osteoclast function and impairment of bone resorption.

Osteoblast

Cell

An osteoblast is a specialized connective tissue-related bone cell responsible for the synthesis and mineralization of bone during the initial bone formation, as well as bone remodeling.

Osteoclast

Cell

Osteoclasts are giant multinuclear bone cells of hematopoietic origin responsible for dissolution and absorption of bone. This function is critical for the maintenance, repair, and remodeling of bones in the vertebral skeleton.

Renal tubular acidosis

Disease

Renal tubular acidosis is a medical condition characterized by metabolic acidosis, which occurs due to defective renal acid excretion.

## Pathway 1

### Osteoclast functional impairment in osteopetrosis (Fig. 6)

#### Incoming signals

Osteopetrosis is a group of rare inherited skeletal disorders characterized by a marked increase in bone density owing to defective bone resorption by osteoclasts, the cells devoted explicitly to this function in the bone tissue. Mutations in the genes involved in the acidification machinery of osteoclasts cause the osteoclast-rich osteopetrosis.

#### Outcome effects

As a result of genetic mutations, the adhesion of osteoclasts to the bone and bone acidification is impaired, leading to a physiological reduction of bone resorption (Del Fattore et al., 2008; Lange et al., 2006; Palagano et al., 2018; Stark and Savarirayan, 2009).

#### Signaling

A carbonic anhydrase II (CAII) deficiency was the first form of osteopetrosis with recognized genetic pathogenesis. The CAII gene encodes the cytoplasmic enzyme that catalyzes the formation of  $\text{H}_2\text{CO}_3$  from  $\text{CO}_2$  and  $\text{H}_2\text{O}$ .  $\text{H}_2\text{CO}_3$  dissociates into  $\text{HCO}_3^-$  and  $\text{H}^+$  ions, and then, the  $\text{H}^+$  ions are extruded by the vacuolar proton-transporting V-ATPase.  $\text{HCO}_3^-$  ions are taken up by a  $\text{Cl}^-/\text{HCO}_3^-$  anion exchanger protein located in the basolateral membrane, thus preventing cytoplasmic alkalinization while providing  $\text{Cl}^-$  ions for the CLCN7/OSTM1  $2\text{Cl}^-/\text{H}^+$  antiporter.

Mutations in the genes encoding the subunit of V-ATPase (T-cell immune regulator 1, *TCIRG1*) and the chloride-specific ion channel chloride voltage-gated channel 7 (*CLCN7*) are associated with severe malignant osteopetrosis (Tolar et al., 2004). Mutations in *TCIRG1* cause defects in the proton-pumping function of the V-ATPase and in vesicle trafficking and fusion within osteoclasts. The *TCIRG1* gene encodes the a3 subunit of the V0 domain of the ATP-dependent vacuolar proton pump V-ATPase. It is mostly expressed in osteoclasts where activity of the V-ATPase is required to achieve the low pH needed for dissolution of the inorganic matrix and the degradation of the organic matrix by acid proteases. This explains the defects in bone mineralization observed with loss function mutations in *TCIRG1*.

The *CLCN7* gene encodes a ubiquitously expressed slowly voltage-gated  $2\text{Cl}^-/\text{H}^+$  antiporter channel located on the membrane of late endosomes and lysosomes. *CLCN7* exchanges chloride ions with

protons, thus cooperating with V-ATPase in the acidification of resorption lacuna and lysosomal vesicles. CLCN7 functions are closely associated with another membrane protein, osteopetrosis-associated transmembrane protein 1 (OSTM1) (Leisle et al., 2011; Qin et al., 2012). The *OSTM1* gene encodes a type I transmembrane protein localized mainly on endosomes and lysosomes. The OSTM1 protein has a highly glycosylated N-terminus, which stabilizes CLCN7 and protects it from lysosomal degradation. Mutations in the *OSTM1* gene that lead to the secretion of a truncated form of OSTM1 have been shown to inhibit osteoclast formation through the downregulation of the PRDM1-NFATc1 cascade (Lange et al., 2006; Shin et al., 2014).

The pleckstrin homology domain-containing family M1 (*PLEKHM1*—with RUN domain-member 1) gene participates in the fusion of autophagosomes and lysosomes, which is required for the clearance of various protein aggregates. It encodes a cytosolic protein implicated in endosomal trafficking pathways through its interaction with the small GTPases RAB7 and ARL8. Loss of *PLEKHM1* impairs vesicle distribution, secretion, and ruffled border formation, thus undermining the resorptive function of osteoclasts (Marwaha et al., 2017; Witwicka et al., 2015).

The fermitin family member 3 (*FERMT3*) gene encodes kindlin-3, a member of the kindlin family that comprises three different focal adhesion proteins involved in integrin activation. Kindlin-3 is an intracellular protein linked to the actin cytoskeleton. It interacts with multiple integrin classes and mediates their adhesive function and inside-out signaling, which in bone is essential for the resorptive activity of osteoclasts. Mutations in the *FERMT3* gene lead to a kindlin-3 protein deficiency, which impairs the ability of osteoclasts to adhere to bone surfaces (Schmidt et al., 2011).

The sorting nexin 10 (*SNX10*) gene encodes a protein that interacts with V-ATPase and regulates its subcellular trafficking. Loss of function mutations in the *SNX10* gene alter the V-ATPase trafficking to the ruffled border, resulting in defective osteoclast function. *SNX10* also plays a role in the trafficking and secretion of matrix metalloprotease nine, which is needed for the degradation of the extracellular matrix (Aker et al., 2012; Zhou et al., 2017).

The TNF superfamily member 11 (*TNFSF11*) gene codes the osteoclastogenic cytokine that, when bound to its receptor TNF receptor superfamily member 11a (*TNFRSF11A*), regulates osteoclast differentiation and their subsequent activation. *TNFRSF11A* trimerization and the recruitment of different adaptor molecules stimulates the downstream activation of several transcriptional factors such as the JUN-FOS/activator protein-1 (AP-1), nuclear factor kappa B (NF- $\kappa$ B), and the nuclear factor of activated T-cell c1 (NFATC1), resulting in osteoclast differentiation, activation, and survival. TNF receptor-associated factor 6 (*TRAF6*) appears to be the most important of the different adaptor molecules recruited when

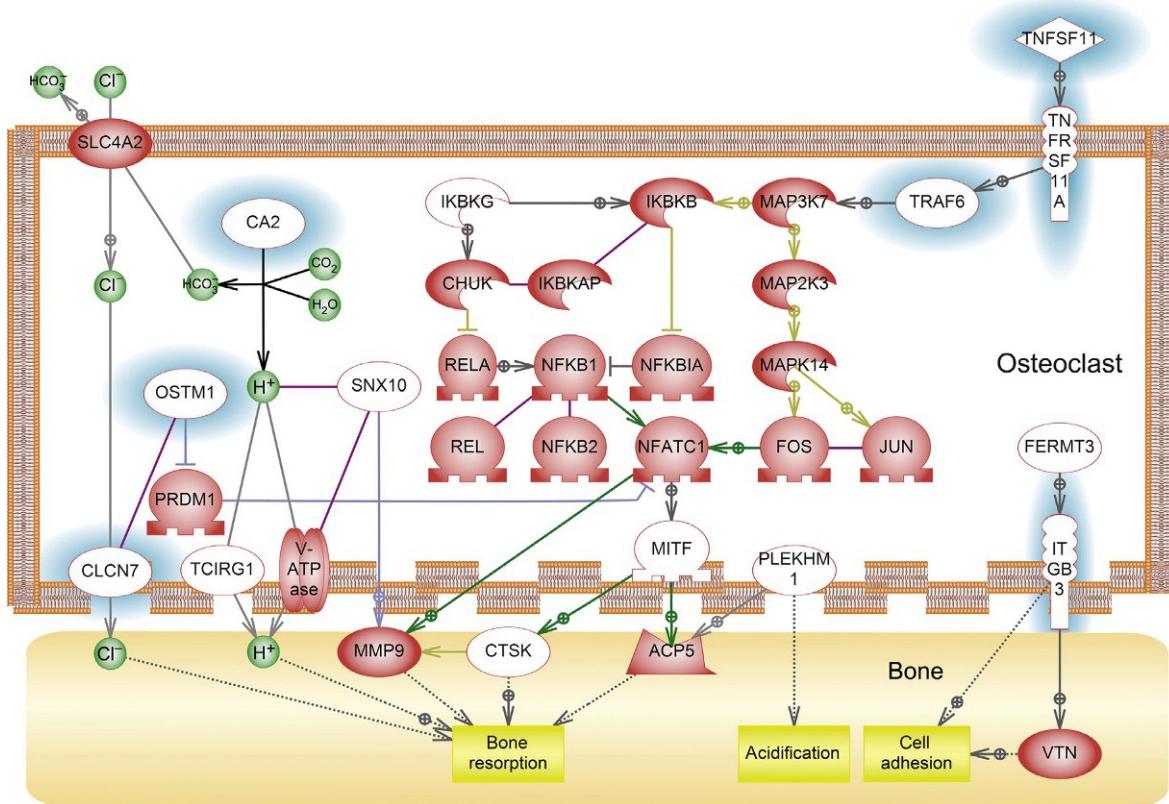
RANKL/RANK binds. *TRAF6* gene inactivation causes severe osteopetrosis ([Guerrini et al., 2008; Liu and Zhang, 2015](#)).

The microphthalmia-associated transcription factor (MITF) has been proposed to act along the RANKL/RANK signaling pathway downstream of NFATC1 in order to amplify NFATC1-dependent osteoclastogenic signals in bone. Several mutations in the *MITF* gene have been associated with osteopetrosis.

The NF- $\kappa$ B pathway involves a number of molecules, which together play a crucial role in regulating gene expression in bone. Hypomorphic mutations in the *IKBKG* gene (which encodes a component of the I $\kappa$ B kinase complex) result in the inhibition of I $\kappa$ B-alfa and the subsequent nuclear translocation of the released RELA/NFKB1 heterodimer. *IKBKG* mutations are associated with osteopetrosis presenting with ectodermal dysplasia and immunodeficiency ([Miot et al., 2017; Roberts et al., 2010](#)).

The *CTSK* gene encodes cathepsin K, a cysteine peptidase of the papain superfamily, which is exploited by osteoclasts in bone matrix degradation, and it is endowed with the unique capacity to cleave collagen molecules at multiple sites. Also, cathepsin K cleaves and activates matrix metalloproteinase nine, which suggests the presence of a protease-signaling network, and it contributes to the regulation of bone modeling by degrading periostin, a matricellular protein of the cortical compartment essential for periosteal bone formation. *CTSK* gene mutations lead to increased bone density in long bones ([Bonnet et al., 2017; Christensen and Shastri, 2015](#)).

## II. Human disease pathways



**FIG. 6** Pathway 1: Osteoclast functional impairment in osteopetrosis.

## Pathway 2

### WNT signaling dysregulation in osteoblasts (Fig. 7)

#### Incoming signals

Osteoblasts regulate osteoclast function through the production of paracrine factors, mainly TNFSF11, which is involved in WNT signaling. Mutations in WNT pathway components cause skeletal abnormalities associated with a lack of TNFSF11.

#### Outcome effects

The lack or TNFSF11 production by osteoblasts results in the impairment of osteoclast activation and the absence of physiological bone resorption. Ultimately, this leads to osteopetrosis.

#### Signaling

The WNT pathway is important for the growth and renewal of bone. The WNT signaling cascade imposes tight regulation on the transcriptional coactivator  $\beta$ -catenin through a complex of proteins including axin 1 (AXIN1), glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), and the adenomatous polyposis coli gene (APC, a WNT signaling pathway regulator). The WNT/beta-catenin pathway is triggered when the WNT ligand binds to Frizzled receptors (FZD) and the subsequent downstream disruption of the beta-catenin degradation complex through the action of heterotrimeric G-proteins along with the phosphoprotein disheveled segment polarity protein 1 (DVL1). This allows beta-catenin to accumulate in the cytosol and bind to members of the lymphoid enhancer binding factor 1 (LEF1)/transcription factor 4 (TCF4) family of transcription factors.

The accumulation of bone mass is normally regulated by WNT signaling and includes stem cell self-renewal, the inhibition of osteocyte apoptosis, and the stimulation of both osteoblast differentiation and proliferation. Defects in WNT pathway components cause skeletal abnormalities. Mutations in the WNT coreceptor low-density lipoprotein receptor-related protein 5 (LRP5) lead to the osteoporosis-pseudoglioma syndrome. *PORCN* mutations are associated with osteopathia striata in Goltz syndrome, while *AMER1* gene mutations are linked to osteopathia striata with cranial stenosis.

OSTM1 has been proposed to also act as an E3 ubiquitin ligase for the heterotrimeric G-protein  $G\alpha i3$  and to potentiate WNT canonical signaling by modulating the interaction with CTNNB1/LEF1. OSTM1 regulates the CTNNB1/LEF1 interaction and causes rare forms of osteopetrosis (Del Fattore et al., 2008; Feigin and Malbon, 2008; Stark and Savarirayan, 2009).

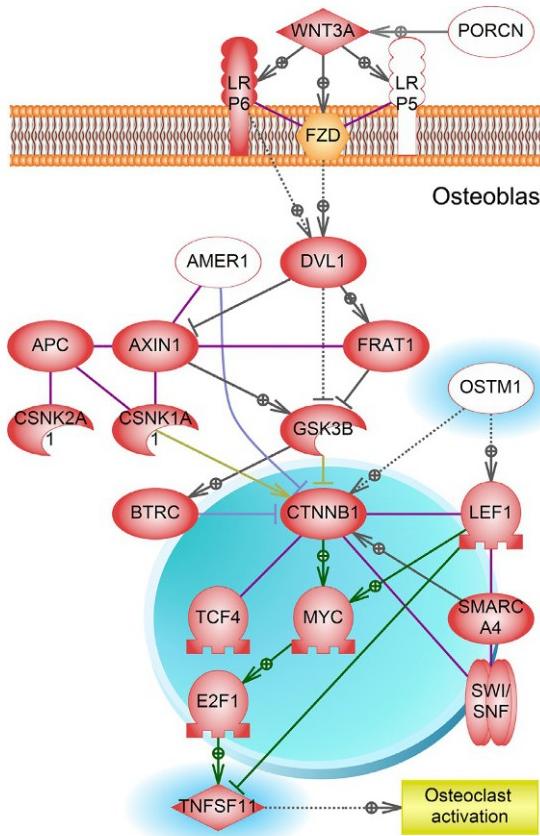


FIG. 7 Pathway 2: WNT signaling dysregulation in osteoblasts.

## Pathway 3

### **Renal tubular acidosis associated with carbonic anhydrase II mutation and osteopetrosis (Fig. 8)**

#### **Incoming signals**

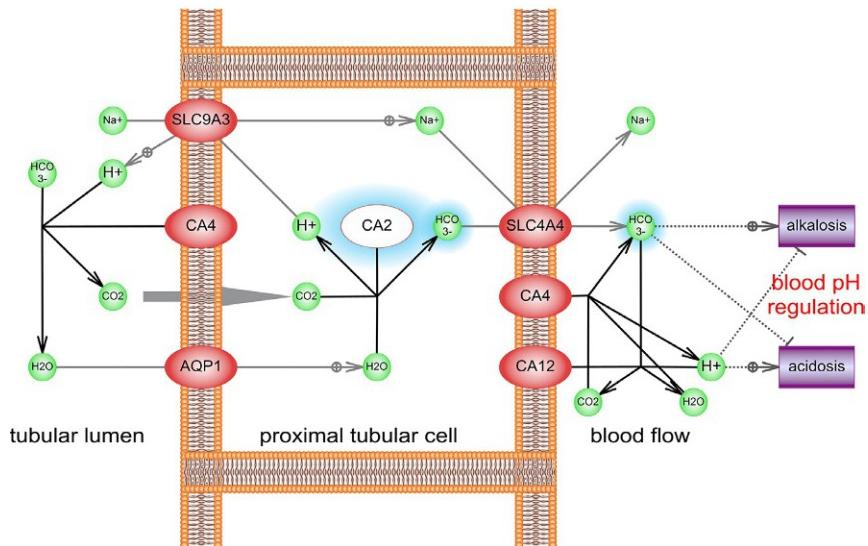
Osteopetrosis with renal tubular acidosis is a rare disorder characterized by osteopetrosis, renal tubular acidosis (RTA), and neurological disorders related to cerebral calcifications. It is caused by mutations in the carbonic anhydrase II (CA2) gene.

#### **Outcome effects**

When CA2 is mutated, the blood acid-base balance is impaired leading to systemic acidosis.

#### **Signaling**

Normally, renal CA2 plays an essential role in the maintenance of the blood acid-base balance. CA2 works in renal proximal tubular cells and catalyzes the reversible hydration of carbon dioxide to form bicarbonate and a proton. In the healthy state, the *CAII* gene encodes the cytoplasmic enzyme that catalyzes the formation of  $\text{H}_2\text{CO}_3$  using  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . It then dissociates into  $\text{HCO}_3^-$  and  $\text{H}^+$  ions. The  $\text{H}^+$  generated is extruded by the V-ATPase, while the  $\text{HCO}_3^-$  is taken up by a  $\text{Cl}^-/\text{HCO}_3^-$  anion exchanger located in the basolateral membrane to prevent cytoplasmic alkalinization. Mutations in the *CAII* gene lead to a decrease in its activity and an inhibition of the formation of  $\text{HCO}_3^-$  ions. As a result, the blood acid-base balance is impaired leading to systemic acidosis ([Del Fattore et al., 2008](#); [Shah et al., 2004](#); [Stark and Savarirayan, 2009](#)). The role of CA2 in osteoclast-mediated bone resorption and its impairment when mutated is shown in [Pathway 1](#).



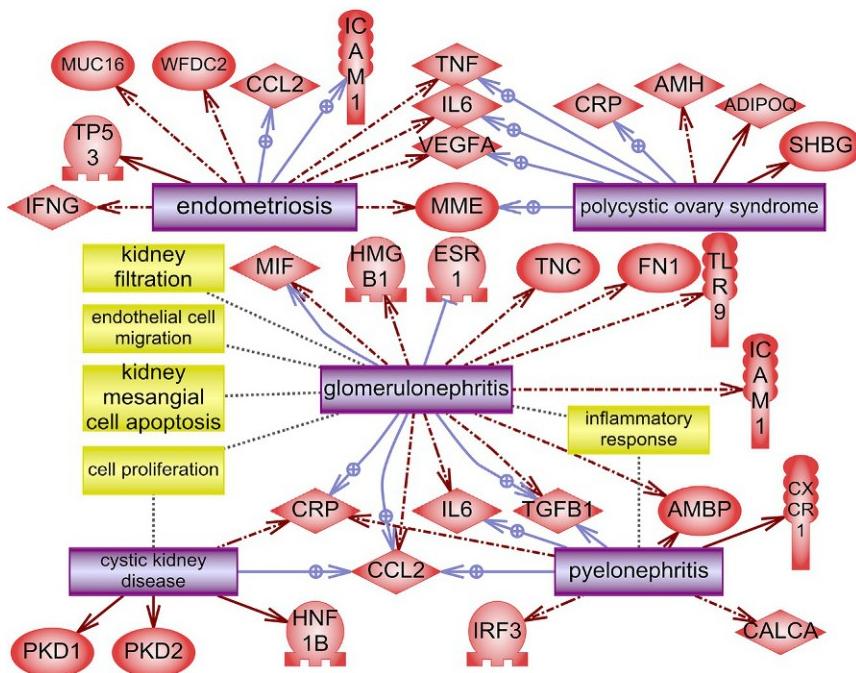
**FIG. 8** Pathway 3: Renal tubular acidosis associated with carbonic anhydrase II mutation and osteopetrosis.

## References

- Disease number #611490, #259730, #259700, #607634, #259720, #166600 (and others) in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code Q78.2. Congenital malformations, deformations and chromosomal abnormalities (Q00-Q99). (ICD-10, <https://icdlist.com>). ICD-11: disease code LD24.10.
- Aker, M., Rouvinski, A., Hashavia, S., Ta-Shma, A., Shaag, A., Zenvirt, S., Israel, S., Weintraub, M., Taraboulos, A., Bar-Shavit, Z., Elpeleg, O., 2012. An SNX10 mutation causes malignant osteopetrosis of infancy. *J. Med. Genet.* 49, 221–226. <https://doi.org/10.1136/jmedgenet-2011-100520>.
- Bonnet, N., Brun, J., Rousseau, J.-C., Duong, L.T., Ferrari, S.L., 2017. Cathepsin K controls cortical bone formation by degrading periostin: periostin mediates cathepsin K bone formation. *J. Bone Miner. Res.* 32, 1432–1441. <https://doi.org/10.1002/jbmr.3136>.
- Christensen, J., Shastri, V.P., 2015. Matrix-metalloproteinase-9 is cleaved and activated by Cathepsin K. *BMC. Res. Notes* 8, <https://doi.org/10.1186/s13104-015-1284-8>.
- Del Fattore, A., Cappariello, A., Teti, A., 2008. Genetics, pathogenesis and complications of osteopetrosis. *Bone* 42, 19–29. <https://doi.org/10.1016/j.bone.2007.08.029>.
- Feigin, M.E., Malbon, C.C., 2008. OSTM1 regulates beta-catenin/Lef1 interaction and is required for Wnt/beta-catenin signaling. *Cell. Signal.* 20, 949–957. <https://doi.org/10.1016/j.cellsig.2008.01.009>.
- Guerrini, M.M., Sobacchi, C., Cassani, B., Abinun, M., Kilic, S.S., Pangrazio, A., Moratto, D., Mazzolari, E., Clayton-Smith, J., Orchard, P., Coxon, F.P., Helfrich, M.H., Crockett, J.C., Mellis, D., Vellodi, A., Tezcan, I., Notarangelo, L.D., Rogers, M.J., Vezzoni, P., Villa, A., Frattini, A., 2008. Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am. J. Hum. Genet.* 83, 64–76. <https://doi.org/10.1016/j.ajhg.2008.06.015>.
- Lange, P.F., Wartosch, L., Jentsch, T.J., Fuhrmann, J.C., 2006. CLC-7 requires Ostm1 as a  $\beta$ -subunit to support bone resorption and lysosomal function. *Nature* 440, 220–223. <https://doi.org/10.1038/nature04535>.
- Leisle, L., Ludwig, C.F., Wagner, F.A., Jentsch, T.J., Stauber, T., 2011. CLC-7 is a slowly voltage-gated  $2\text{Cl}^-(\text{-})/\text{1H}^+(\text{-})$ -exchanger and requires Ostm1 for transport activity. *EMBO J.* 30, 2140–2152. <https://doi.org/10.1038/embj.2011.137>.
- Liu, W., Zhang, X., 2015. Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL)/RANK/osteoprotegerin system in bone and other tissues (Review). *Mol. Med. Rep.* 11, 3212–3218. <https://doi.org/10.3892/mmr.2015.3152>.
- Marwaha, R., Arya, S.B., Jagga, D., Kaur, H., Tuli, A., Sharma, M., 2017. The Rab7 effector PLEKHM1 binds Arl8b to promote cargo traffic to lysosomes. *J. Cell Biol.* 216, 1051–1070. <https://doi.org/10.1083/jcb.201607085>.
- Miot, C., Imai, K., Imai, C., Mancini, A.J., Kucuk, Z.Y., Kawai, T., Nishikomori, R., Ito, E., Pellier, I., Dupuis Girod, S., Rosain, J., Sasaki, S., Chandrasekaran, S., Pachlopnik Schmid, J., Okano, T., Colin, E., Olaya-Vargas, A., Yamazaki-Nakashimada, M., Qasim, W., Espinosa Padilla, S., Jones, A., Krol, A., Cole, N., Jolles, S., Bleesing, J., Vraetz, T., Gennery, A.R., Abinun, M., Güngör, T., Costa-Carvalho, B., Condino-Neto, A., Veys, P., Holland, S.M., Uzel, G., Moshous, D., Neven, B., Blanche, S., Ehl, S., Döfing, R., Patel, S.Y., Puel, A., Bustamante, J., Gelfand, E.W., Casanova, J.-L., Orange, J.S., Picard, C., 2017. Hematopoietic stem cell transplantation in 29 patients hemizygous for hypomorphic *IKBKG*/NEMO mutations. *Blood* 130, 1456–1467. <https://doi.org/10.1182/blood-2017-03-771600>.
- Palagano, E., Menale, C., Sobacchi, C., Villa, A., 2018. Genetics of osteopetrosis. *Curr. Osteoporos. Rep.* 16, 13–25. <https://doi.org/10.1007/s11914-018-0415-2>.
- Qin, A., Cheng, T.S., Pavlos, N.J., Lin, Z., Dai, K.R., Zheng, M.H., 2012. V-ATPases in osteoclasts: structure, function and potential inhibitors of bone resorption. *Int. J. Biochem. Cell Biol.* 44, 1422–1435. <https://doi.org/10.1016/j.biocel.2012.05.014>.

- Roberts, C.M.L., Angus, J.E., Leach, I.H., McDermott, E.M., Walker, D.A., Ravenscroft, J.C., 2010. A novel NEMO gene mutation causing osteopetrosis, lymphoedema, hypohidrotic ectodermal dysplasia and immunodeficiency (OL-HED-ID). *Eur. J. Pediatr.* 169, 1403–1407. <https://doi.org/10.1007/s00431-010-1206-7>.
- Schmidt, S., Nakchbandi, I., Ruppert, R., Kawelke, N., Hess, M.W., Pfaller, K., Jurdic, P., Fässler, R., Moser, M., 2011. Kindlin-3-mediated signaling from multiple integrin classes is required for osteoclast-mediated bone resorption. *J. Cell Biol.* 192, 883–897. <https://doi.org/10.1083/jcb.201007141>.
- Shah, G.N., Bonapace, G., Hu, P.Y., Strisciuglio, P., Sly, W.S., 2004. Carbonic anhydrase II deficiency syndrome (osteopetrosis with renal tubular acidosis and brain calcification): novel mutations in CA2 identified by direct sequencing expand the opportunity for genotype-phenotype correlation. *Hum. Mutat.* 24, 272. <https://doi.org/10.1002/humu.9266>.
- Shin, B., Yu, J., Park, E.-S., Choi, S., Yu, J., Hwang, J.M., Yun, H., Chung, Y.-H., Hong, K.S., Choi, J.-S., Takami, M., Rho, J., 2014. Secretion of a truncated osteopetrosis-associated transmembrane protein 1 (OSTM1) mutant inhibits osteoclastogenesis through down-regulation of the B lymphocyte-induced maturation protein 1 (BLIMP1)-nuclear factor of activated T cells c1 (NFATc1) axis. *J. Biol. Chem.* 289, 35868–35881. <https://doi.org/10.1074/jbc.M114.589614>.
- Stark, Z., Savarirayan, R., 2009. Osteopetrosis. *Orphanet J. Rare Dis.* 4, 5. <https://doi.org/10.1186/1750-1172-4-5>.
- Tolar, J., Teitelbaum, S.L., Orchard, P.J., 2004. Osteopetrosis. *N. Engl. J. Med.* 351, 2839–2849. <https://doi.org/10.1056/NEJMra040952>.
- Witwicka, H., Jia, H., Kutikov, A., Reyes-Gutierrez, P., Li, X., Odgren, P.R., 2015. TRAFD1 (FLN29) interacts with Plekhn1 and regulates osteoclast acidification and resorption. *PLoS One* 10, e0127537. <https://doi.org/10.1371/journal.pone.0127537>.
- Zhou, C., Wang, Y., Peng, J., Li, C., Liu, P., Shen, X., 2017. SNX10 plays a critical role in MMP9 secretion via JNK-p38-ERK signaling pathway. *J. Cell. Biochem.* 118, 4664–4671. <https://doi.org/10.1002/jcb.26132>.

# Diseases of the genitourinary system



## OUTLINE

Pyelonephritis	572
Glomerulonephritis	582
Polycystic kidney disease	594
Polycystic ovary syndrome	603
Endometriosis	613

Diseases of the genitourinary system constitute a large group of disorders specific to certain sexual and age groups. This chapter focuses on the most common diseases of the kidney and of the female reproductive system.

An extended asymptomatic period is a distinctive feature of many urogenital disorders. Many genitourinary system diseases can become life-threatening conditions because early symptoms remain unnoticed.

Urogenital system infections, congenital pathologies, and functional problems provoked by noninfectious factors are the leading causes of genitourinary system disorders. Infections caused by bacteria, viruses, fungi, and parasites can be sexually transmitted, transferred with blood or lymph flow from other infectious foci within the body, or evolve as a result of poor hygiene.

Kidney disorders—pyelonephritis, glomerulonephritis, and polycystic kidney disease (PKD)—are also included in this chapter. Female reproductive system disorders—polycystic ovary syndrome and endometriosis—are also included in this chapter.

Pyelonephritis is a nonspecific inflammation of kidney tubules that usually begins as a bladder or urethral infection that then spreads into the kidneys. Severe pyelonephritis can be life threatening. Acute pyelonephritis is quite common, especially among young women, but it can affect people of any age and sex. Chronic pyelonephritis may cause chronic kidney failure.

Glomerulonephritis is a group of morphologically heterogeneous immune-mediated diseases. Glomerulonephritis is the third leading cause of chronic kidney failure among all renal diseases.

Polycystic kidney disease (PKD) is relatively rare, but it is the most common inherited kidney pathology and one of the leading causes of kidney failure. PKD is characterized by the development of fluid-filled cysts in the kidney.

Polycystic ovary syndrome (PCOS) and endometriosis are among the most common causes of infertility in women. Polycystic ovary syndrome is characterized by pathological changes of the endocrine system and of ovarian function, which usually leads to an absence of ovulation. In endometriosis the layer of tissue that usually covers the inside of the uterus (the endometrium) tends to grow outside of it. The main symptoms are pelvic pain and infertility. Notably, about 25% of women with endometriosis have no symptoms except for infertility.

## CHAPTER

## 13.1

## Pyelonephritis

Pyelonephritis is a nonspecific inflammation of the renal calyces, pelvis, and parenchyma.

Pyelonephritis is an ascending infection of a bacterial pathogen infecting the renal pelvis and kidney. (*Ferri and Ferri, 2018*).

Pyelonephritis is the most common kidney disease in all age groups. Women are more likely to develop this disorder. Many cases of pyelonephritis result as complications of infections in the bladder, urethra, or prostate in men in which the bacteria migrate to the kidneys. Antibiotic-resistant bacteria appear in hospitalized patients with abiding urinary catheters and may cause pyelonephritis. Also, pathogen penetration into the kidney with acute pyelonephritis can occur hematogenously from any infection locus in the body. The most common pathogens that cause pyelonephritis are *Escherichia coli*; *Proteus*; and, less often, *Pseudomonas aeruginosa*, *Staphylococcus*, *Klebsiella*, and other microbes associated with this disease. For the development of pyelonephritis, pathogens alone are often insufficient. Structural abnormalities of the urinary tract, kidney stones, urine flow obstructions, and other common factors such as improper immunological reactivity and diabetes mellitus predispose pyelonephritis.

Clinical manifestations of pyelonephritis include high fever, pain in the back or flank, nausea, and vomiting. Older individuals may also suffer from unexplained anorexia, other organ system decompensation, and a generalized worsening of their condition. In recent years the proportion of asymptomatic and atypical variants of pyelonephritis has increased. Pyelonephritis can cause subsequent kidney scarring and chronic renal failure and can even be life threatening.

If uropathogens penetrate in the kidney, they attach to urothelium, move on it, and damage it. Different bacteria have their own mechanisms of adhesion, colonization, damage causation, and avoidance of normal protective factors. Despite this, kidney cells recognize pathogens by means of toll-like receptors and provide an immune response via the production of cytokines that recruit and activate immune cells:

**Pathway 1.** The role of *toll-like receptors in pyelonephritis* (Fig. 1).

The enhanced production of proinflammatory cytokines causes neutrophil activation and the failure of endothelial regulatory functions.

**Pathway 2.** The role of *endothelial cells in pyelonephritis* (Fig. 2).

During inflammation, cytokines and structural-functional impairments of the microcirculation and the tubular epithelium lead to extracellular matrix degradation and kidney fibrosis.

**Pathway 3.** The role of *interstitial fibroblasts in pyelonephritis* (Fig. 3).

## Key cellular contributors and processes

Fibrosis

Process

Fibrosis is the development of excessive fibrous connective tissue and accumulation of extracellular matrix proteins in an organ or tissue, which occurs as reparative response to tissue damage. Fibrosis leads to scarring and thickening of the affected tissue and disrupts its function.

Interstitial edema

Process

Interstitial edema is a condition of abnormally large interstitial fluid (IF) volume. IF is a solution that fills spaces between cells within tissues (interstitial spaces).

Toll-like receptors

Protein or gene

Toll-like receptors belong to a family of membrane proteins that can directly bind microbial molecules or proteins and initiate the innate immune response.

## Pathway 1

### The role of toll-like receptors in pyelonephritis (Fig. 1)

#### Incoming signals

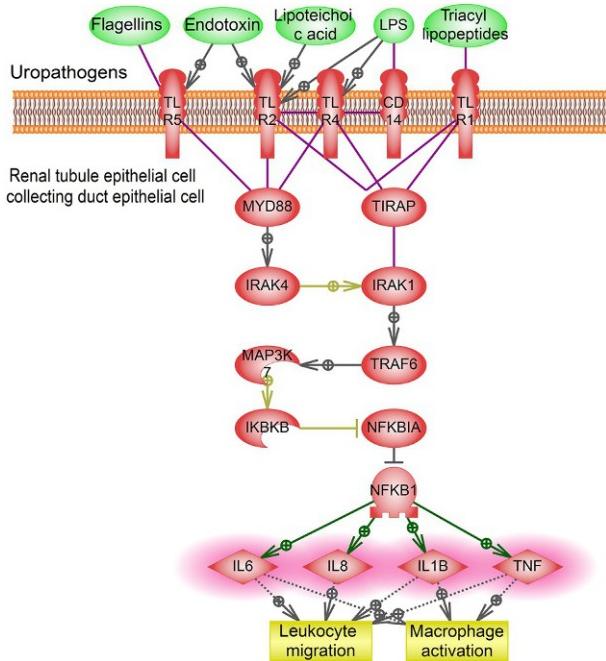
During pyelonephritis, pathogenic bacteria are identified by the toll-like receptors (TLR) of the renal tubule and collecting duct epithelial cells. This is a class of cellular receptors that recognize bacterial proteins and components of the bacterial cell wall such as the flagellins, endotoxin, lipoteichoic acid, lipopolysaccharides, triacyl lipopeptides, and structures of microorganisms, and in turn, they launch the production of cytokines.

#### Outcome effects

Cytokines recruit inflammatory neutrophils (see [Pathway 2](#)) and activate tissue macrophages, which together provide an immune response.

#### Signaling

The toll-like receptors TLR1, TLR2, TLR4, and TLR5 interact with the adaptor protein MYD88 that triggers the sequential activation of the IRAK4 and IRAK1 kinases and the signal transduction protein TRAF6. TRAF6 then activates MAP3K7, which leads to the activation of the transcription factor NFKB1 through successive phosphorylation events and blocking of its inhibitor NFKBIA. Active NFKB1 induces the production of the proinflammatory cytokines IL-6, CXCL8 (IL-8), IL-1B, and TNF ([Chassin et al., 2006](#); [El-Achkar and Dagher, 2006](#); [Spencer et al., 2014](#); [Vandewalle, 2008](#)).



**FIG. 1** Pathway 1: The role of toll-like receptors in pyelonephritis.

## Pathway 2

### The role of endothelial cells in pyelonephritis (Fig. 2)

#### Incoming signals

In a healthy state the endothelium regulates the migration of immune cells into tissues, vascular permeability, and tone, and it prevents blood from coagulating. Infection leads to the enhanced production of TNF, IL-1B, and IL-6 in kidney epithelial cells, which leads to the recruitment and activation of neutrophils, the primary immune cells involved in the inflammatory response. However, during inflammation, the endothelial cell may lose its ability to regulate some of its normal functions.

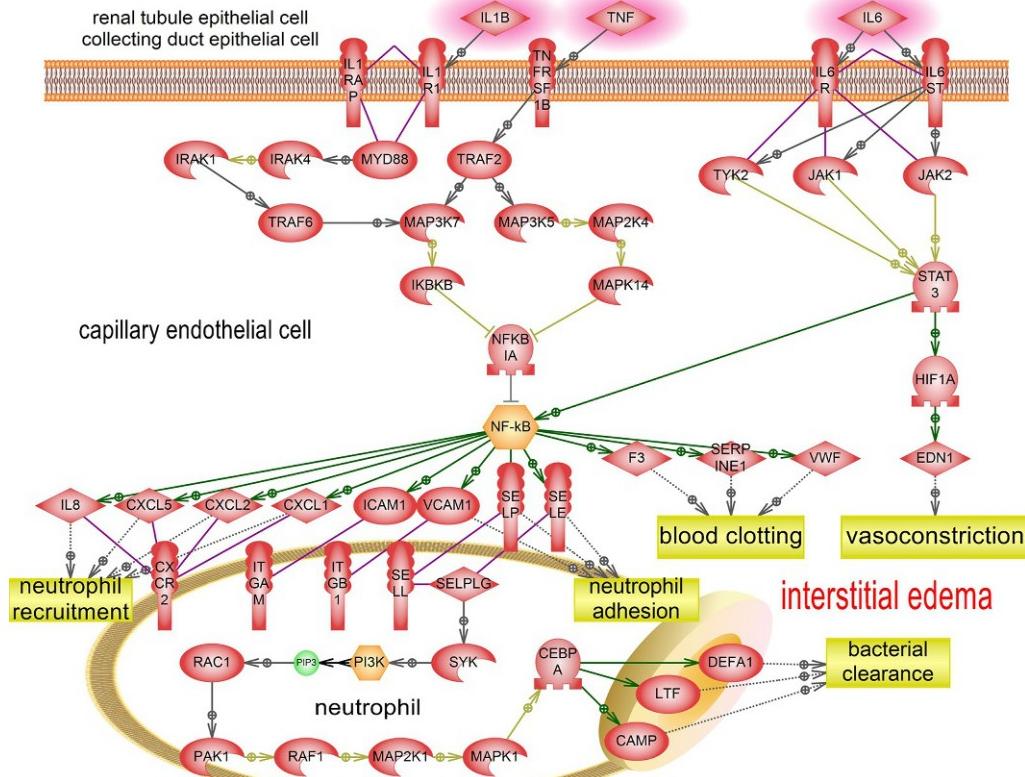
#### Outcome effects

Excessive proinflammatory cytokine production initiates blood clotting and the constriction of microcirculatory vessels. These events are accompanied by a local oxygen deficiency, interstitial edema, and further tissue damage. Recruited neutrophils clear tissues of bacteria and attract other immune cells that support inflammation.

#### Signaling

TNF and IL-1B activate their receptors (TNFRSF1B and IL-1R1) via the associated factor TRAF2 and the adaptor protein MYD88 (see Signaling of [Pathway 1](#)). Together, they mediate signal transduction via the MAPK cascade that results in NFKB activation. Activated NFKB induces the production of the chemokines CXCL8, CXCL5, CXCL2, CXCL1, the intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), and the selectins E and P (SELE and SELP), which are involved in neutrophil recruitment and adhesion. Inducing production of the plasminogen activator inhibitor (SERPINE1), Willebrand factor (VWF), and coagulation factor III (F3) leads to blood clotting in microcirculatory vessels. The activated IL-6 receptors IL-6R and IL-6ST interact with the Janus kinases 1 and 2 (JAK1 and JAK2) and tyrosine kinase 2 (TYK2), which activate the signal transducer and activator of transcription 3 (STAT3) and increase levels of endothelin 1 (EDN1) via the transcription factor HIF1A. EDN1 causes vasoconstriction of peritubular capillaries. Chemokines, selectins, and adhesion molecules of both neutrophils and endothelial cells interact with each other to result in neutrophil activation and the secretion of antimicrobial proteins and peptides such as alpha-defensin (DEFA1), cathelicidin antimicrobial peptide (CAMP), and lactoferrin (LTF), which are required for bacterial clearance ([Choong et al., 2015; Spencer et al., 2014; Sprague and Khalil, 2009; Webb and Brenchley, 2004](#)).

## II. Human disease pathways



**FIG. 2** Pathway 2: The role of endothelial cells in pyelonephritis.

## Pathway 3

### The role of interstitial fibroblasts in pyelonephritis (Fig. 3)

#### Incoming signals

Damage to the kidney interstitium (i.e., the connective tissue forming the organ stroma) is crucial in the development of pyelonephritis. Interstitial fibroblasts produce the extracellular matrix, and they regulate the spatial relationships between kidney components. The tubular epithelium, endothelium, and interstitial cells cooperate within an integrated microenvironment. Increased expression levels of TGFB1, TNF, PDGFB, and FGF2 in renal and immune cells, due to inflammation, can lead to extracellular matrix degradation and fibrosis (Boor et al., 2014; Loeffler and Wolf, 2014; Vielhauer and Mayadas, 2007).

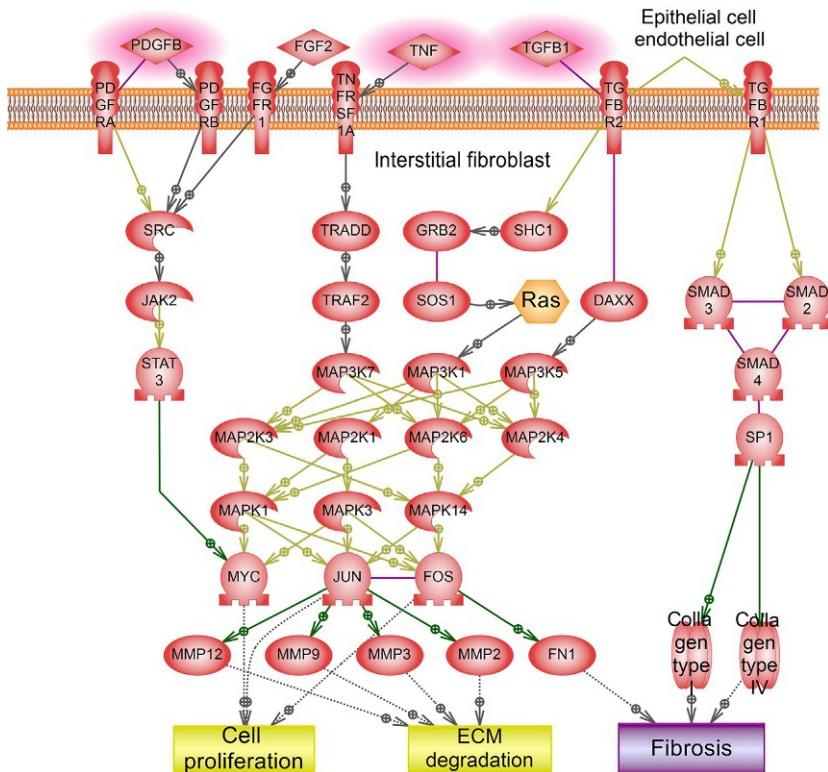
#### Outcome effects

Structural or functional impairment of the extracellular matrix and damage to microcirculation vessels and the tubular epithelium result in disturbances of tubulointerstitial interactions and the replacement of functional tissue with dysfunctional tissue. Interstitial expansion and the development of fibrosis are critical steps in the progression of pyelonephritis to chronic renal disease.

#### Signaling

TGFB1 and TNF interact with their receptors (TGFBR2 and TNFRSF1A, respectively) and, through the adaptor proteins DAXX and TRADD, mediate the MAPK cascade leading to the activation of the transcriptional factors JUN, FOS, and MYC. PDGFB and FGF2 bind to their respective receptors PDGFRA, PDGFRB, and FGFR1 to stimulate JAK2/STAT3/MYC signaling. Transcription factors induce fibroblast proliferation and the production of matrix metallopeptidases (MMP2, MMP3, MMP9, and MMP12) that participate in extracellular matrix degradation.

TGFBR1, phosphorylated by TGFBR2, interacts with SMAD2, SMAD3, and SMAD4 to form a heterotetrameric complex, which translocates into the nucleus and activates transcription factor SP1. SP1 in turn promotes the synthesis of collagen types I and IV, which are involved in the progression of fibrosis (Djudjaj and Boor, 2018; Eddy, 2005; Gewin, 2018; Ucero et al., 2010).



**FIG. 3** Pathway 3: The role of interstitial fibroblasts in pyelonephritis.

## References

- ICD-10: disease code N10-N11. Diseases of the genitourinary system (N00-N99). (ICD-10, <https://icdlist.com>). ICD-11: disease code GB51/GB55/GB56.
- Boor, P., Ostendorf, T., Floege, J., 2014. PDGF and the progression of renal disease. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* 29 (Suppl. 1), i45–i54. <https://doi.org/10.1093/ndt/gft273>.
- Chassin, C., Goujon, J.-M., Darche, S., du Merle, L., Bens, M., Cluzeaud, F., Werts, C., Ogier-Denis, E., Le Bouguénec, C., Buzoni-Gatel, D., Vandewalle, A., 2006. Renal collecting duct epithelial cells react to pyelonephritis-associated *Escherichia coli* by activating distinct TLR4-dependent and -independent inflammatory pathways. *J. Immunol.* 177, 4773–4784.
- Choong, F.X., Antypas, H., Richter-Dahlfors, A., 2015. Integrated pathophysiology of pyelonephritis. *Microbiol. Spectr.* 3. <https://doi.org/10.1128/microbiolspec.UTI-0014-2012>.
- Djudjaj, S., Boor, P., 2018. Cellular and molecular mechanisms of kidney fibrosis. *Mol. Asp. Med.* <https://doi.org/10.1016/j.mam.2018.06.002>.
- Eddy, A.A., 2005. Progression in chronic kidney disease. *Adv. Chronic Kidney Dis.* 12, 353–365. <https://doi.org/10.1053/j.ackd.2005.07.011>.
- El-Achkar, T.M., Dagher, P.C., 2006. Renal Toll-like receptors: recent advances and implications for disease. *Nat. Clin. Pract. Nephrol.* 2, 568–581. <https://doi.org/10.1038/ncpneph0300>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Gewin, L.S., 2018. Renal fibrosis: primacy of the proximal tubule. *Matrix Biol. J. Int. Soc. Matrix Biol.* 68–69, 248–262. <https://doi.org/10.1016/j.matbio.2018.02.006>.
- Loeffler, I., Wolf, G., 2014. Transforming growth factor- $\beta$  and the progression of renal disease. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* 29 (Suppl. 1), i37–i45. <https://doi.org/10.1093/ndt/gft267>.
- Spencer, J.D., Schwaderer, A.L., Becknell, B., Watson, J., Hains, D.S., 2014. The innate immune response during urinary tract infection and pyelonephritis. *Pediatr. Nephrol.* 29, 1139–1149. <https://doi.org/10.1007/s00467-013-2513-9>.
- Sprague, A.H., Khalil, R.A., 2009. Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochem. Pharmacol.* 78, 539–552. <https://doi.org/10.1016/j.bcp.2009.04.029>.
- Ucer, A.C., Gonçalves, S., Benito-Martin, A., Santamaría, B., Ramos, A.M., Berzal, S., Ruiz-Ortega, M., Egido, J., Ortiz, A., 2010. Obstructive renal injury: from fluid mechanics to molecular cell biology. *Open Access J. Urol.* 2, 41–55.
- Vandewalle, A., 2008. Toll-like receptors and renal bacterial infections. *Chang Gung Med. J.* 31, 525–537.
- Vielhauer, V., Mayadas, T.N., 2007. Functions of TNF and its receptors in renal disease: distinct roles in inflammatory tissue injury and immune regulation. *Semin. Nephrol.* 27, 286–308. <https://doi.org/10.1016/j.semnephrol.2007.02.004>.
- Webb, N.J.A., Brenchley, P.E.C., 2004. Cytokines and cell adhesion molecules in the inflammatory response during acute pyelonephritis. *Nephron Exp. Nephrol.* 96, e1–e6. <https://doi.org/10.1159/000075570>.

## CHAPTER

## 13.2

## Glomerulonephritis

Glomerulonephritis is a group of immune kidney diseases characterized by primary lesions of the glomeruli. In more than half the cases, the etiology of the disease remains unknown. Glomerulonephritis is associated with certain infections (e.g., it often occurs after streptococcal infection), drug consumption, and systemic disorders.

Acute glomerulonephritis (acute nephritic syndrome) is an immunologically mediated inflammation of the filtering unit of the kidney called the glomerulus. The inflammation may result in damage to the basement membrane, mesangium, and/or capillary endothelium. (*Ferri and Ferri, 2018*).

Symptoms of glomerulonephritis may include edema due to protein loss with urine, high blood pressure, hematuria, and proteinuria. The disease affects both adults and children and many forms of acute glomerulonephritis tend to progress into chronic diseases. Chronic glomerulonephritis has a long course with periods of remission and relapse but after several years it leads to glomerulosclerosis and chronic renal failure. Glomerulonephritis is a severe illness that can be life threatening. It accounts for about 25% of end-stage renal disease cases.

When infections develop in the body, immune cells begin to produce antibodies that bind with exogenous antigens and form immune complexes (antigen-antibody complexes). If not neutralized in the peripheral bloodstream, complexes are transported to the kidney and deposited in the basement membrane and mesangial cells of glomeruli. This event activates the kidney complement system that forms membrane attack complex (MAC) and produces chemoattractants to recruit additional immune cells. MAC attacks the membrane of any cell and destroys it. Recruited neutrophils englobe immune complexes and release cytokines, which damage glomerular cells:

**Pathway 1. The role of complement in glomerulonephritis (Fig. 4).**

Further progression of the disease invokes the excessive release of cytokines and growth factors, which cause endothelial injury, podocyte and mesangial cell (specialized cells of the glomeruli that provide structural

support and regulate filtration rate) dysfunction, and glomerular filtration damage.

**Pathway 2.** *Podocyte dysfunction in glomerulonephritis* (**Fig. 5**).

**Pathway 3.** *Mesangial cell dysfunction in glomerulonephritis* (**Fig. 6**).

Ultimately, chronic glomerulonephritis leads to the development of glomerulosclerosis, retention of uremic toxins, and cachexia (extreme exhaustion and wasting).

## Key cellular contributors and processes

Basement membrane

Anatomic structure

The basement membrane is a thin protective layer of extracellular matrix that underlies or surrounds epithelial or endothelial cells and separates them from other cells, for example, connective tissue cells.

Complement system

System of organism

The complement system refers to a group of small proteins that “complement” the ability of the antibody system to eliminate cellular pathogens. The complement system proteins, produced by the liver and circulating in the blood as inactive precursors, promote inflammation and attack the pathogen's plasma membrane.

Fibrosis

Process

Fibrosis is the development of excessive fibrous connective tissue and accumulation of extracellular matrix proteins in an organ or tissue, which occurs as a reparative response to tissue damage. Fibrosis leads to scarring and thickening of the affected tissue and disrupts its function.

Hematuria

Process

Hematuria is a condition in which blood is found in the urine. Hematuria can be gross (visible discoloration of the urine) or microscopic (invisible by the naked eye) and can be caused by various problems with the kidneys or urinary tract.

Mesangial cells

Cell

Mesangial cells are contractile cells in the kidney that make up the mesangium of the glomerulus. The primary function of mesangial cells is to remove trapped residues and aggregated protein from the glomerular basement membrane, thus keeping the filter free of debris.

Podocytes

Cell

Podocytes are highly specialized epithelial cells in the visceral layer of the kidney Bowman's capsule (glomerulus) attached to the basement membrane of the capillaries via cytoplasmic pedicles (footlike projections). Podocytes participate in the formation of glomerular filtration barrier.

**Proteinuria****Process**

Proteinuria is the presence of larger than normal amounts of protein in the urine and can be a sign of disease.

## Pathway 1

### The role of complement in glomerulonephritis (Fig. 4)

#### Incoming signals

Glomerulonephritis is associated with mutations in the genes encoding proteins of the complement system such as C3-convertase; complement component 3 (C3); the complement factors B, H, and I (CFB, CFH, and CFI); and the complement regulatory factors 1 and 5 (CFHR1 and CFHR5) (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

The genetic changes related to glomerulonephritis increase activation levels of the complement system. The main purpose of complement activation is to remove invading pathogens and purify cells. This is achieved directly either through the formation of the membrane attack complex (MAC) or indirectly by opsonization and phagocytosis stimulation.

#### Outcome effects

The final morphological pathologies resulting from these molecular mechanisms cause damage to glomeruli by inflammatory cytokines (see [Pathway 2](#)) and MAC action. MAC forms a transmembrane channel that in turn results in an osmotic imbalance that leads to cell lysis.

#### Signaling

Once initiated the complement system forms C1-complexes (C1QA-C1R-C1S) when the C1QA component binds to the Fc region of IgG or IgM. The sequential cleavage of the C4B and C2 components by the C1-complex leads to the assembly of C3-convertase (i.e., the C4BC2A-complex). C3-convertase cleaves the C3 component into C3A and C3B. C3B binds with the C4B2A-complex to make C5-convertase (i.e., the C4BC2AC3B-complex), which cleaves C5 into C5A and C5B. The C5B, C6, C7, C8, and C9 components form MAC via the classical complement pathway. Different complement factors and regulatory factors contribute to the formation and stabilization of the convertases. Products of C3 and C5 activation are recognized by complement receptors C3AR1 and C5AR1 present on neutrophils and lead to phagocytosis of the opsonized target. At the same time, immune complexes are deposited in the glomerular basement membrane and continue to damage it ([Barbour et al., 2013](#); [Fearn and Sheerin, 2015](#); [Masani et al., 2014](#); [Zipfel et al., 2015](#)).

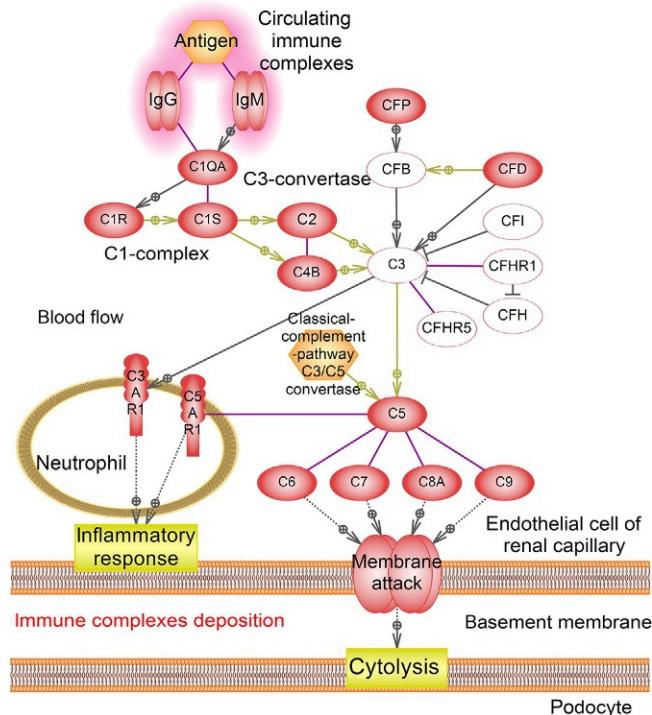


FIG. 4 Pathway 1: The role of complement in glomerulonephritis.

## Pathway 2

### Podocyte dysfunction in glomerulonephritis (Fig. 5)

#### Incoming signals

One of the main podocyte functions is the formation of filtration slits through which small molecules pass and protein molecules are retained. Increased levels of TGFB1 and TNF make a significant contribution to podocyte dysfunction (Kronbichler et al., 2016; Loeffler and Wolf, 2014). As the disease progresses, glomerular hypertension develops. High glomerular capillary pressure may stimulate the production of angiotensin II (AGT) in kidney to further cause podocyte injury.

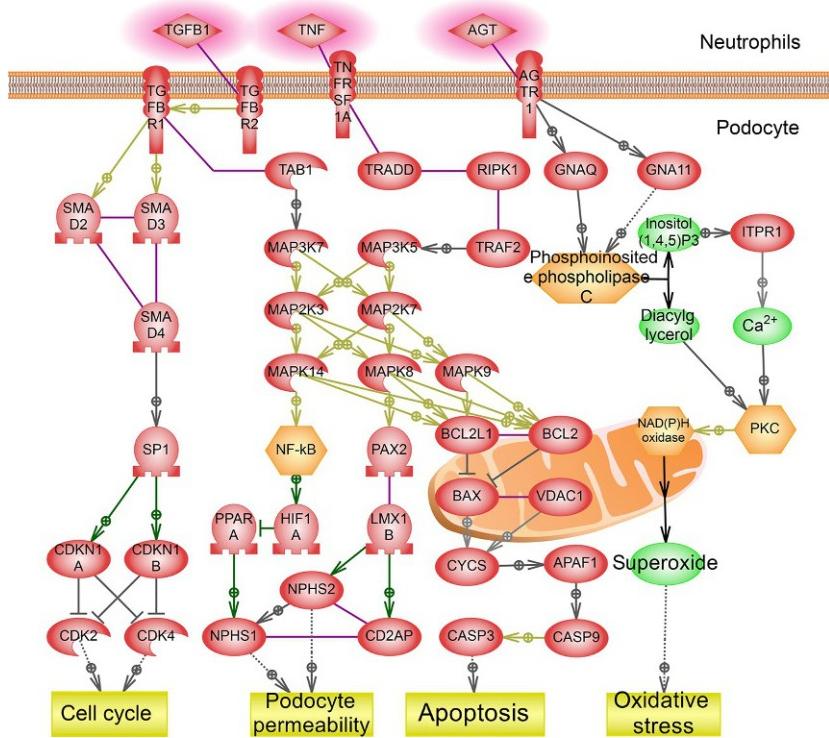
#### Outcome effects

Podocytes are highly differentiated cells that make their proliferation difficult. Pathogenetic factors induce podocyte apoptosis or the sustained expression of cell cycle inhibitors while causing podocyte injury as a result of oxidative stress or the improper regulation of the synthesis of specific proteins. Disturbing podocyte function or induction of their death leads to a reduction of glomerular filtration rate and proteinuria (a condition in which elevated amounts of protein are present in urine).

#### Signaling

AGT binds with its receptor AGTR1, and through the G proteins, GNA11 and GNAQ activate phosphoinositide phospholipase C, which in turn generates inositol 1,4,5-trisphosphate that mediates  $\text{Ca}^{2+}$  release from endoplasmic reticulum stores. PKC, activated by  $\text{Ca}^{2+}$ , phosphorylates cytosolic NADPH oxidase that itself generates superoxide inducing oxidative stress. TNF and TGFB1 bind to their respective receptors (TNFRSF1A and TGFBR2 phosphorylates TGFBR1) and mediate the MAPK cascade, which leads to the activation of the transcription factors NF- $\kappa$ B and PAX2. Terminal MAPKs phosphorylate the antiapoptotic regulators BCL2 and BCL2L1 and mediate their degradation to prevent suppression of the apoptotic activator BAX. BAX interacts with the mitochondrial channel protein VDAC1, which leads to the formation of a mitochondrial transition pore and the release of cytochrome c from the mitochondria into the cytoplasm. Cytochrome c binds to APAF1, which in turn activates caspase-9 to form an apoptosome leading to subsequent activation of apoptotic caspases. TGFBR1 interacts with SMAD2, SMAD3, and SMAD4 to form a heterotetrameric complex, which translocates into the nucleus and cooperates

with the transcription factor SP1. SP1 promotes expression of the genes encoding cyclin-dependent kinase inhibitors (CDKN1A and CDKN1B), which in turn inhibit activity of the cyclin-dependent kinases 2,3 that are required for cell cycle progression and cellular proliferation. NF- $\kappa$ B and PAX2 regulate the expression the genes encoding nephrin (NPHS1) and podocin (NPHS2) that form podocyte filtration slits and control their permeability (Lee, 2012; Loeffler and Wolf, 2014; Shankland, 2006; Wiggins, 2007).



**FIG. 5** Pathway 2: Podocyte dysfunction in glomerulonephritis.

## Pathway 3

### Mesangial cell dysfunction in glomerulonephritis (Fig. 6)

#### Incoming signals

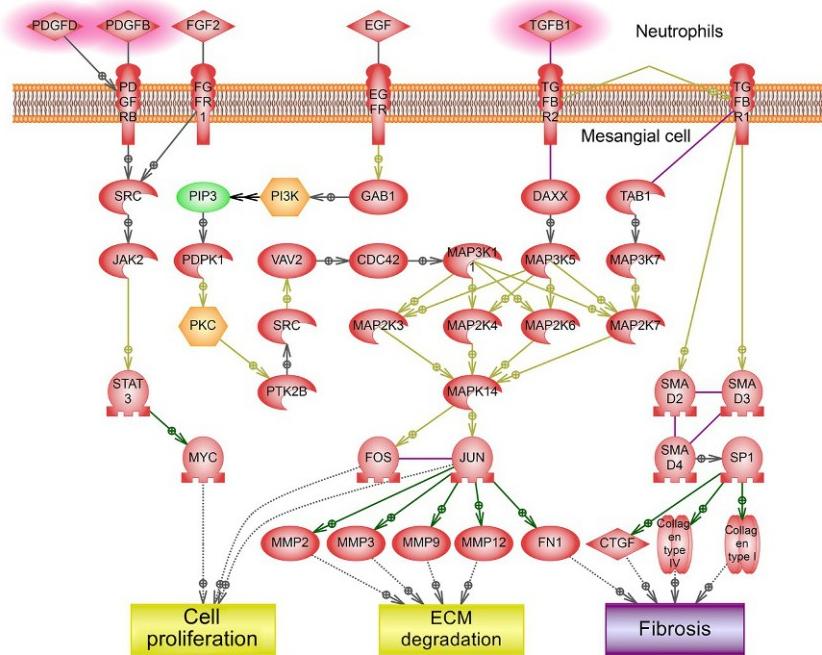
Mesangial cells are located in the intercapillary space and regulate glomerular filtration rate. Overexpressed PDGFB, PDGFD, TGF $\beta$ 1, FGF2, and EGF can stimulate mesangial cells to become matrix-producing cells with the concomitant loss of their main function (Boor et al., 2014; Djudjaj and Boor, 2018).

#### Outcome effects

Mesangial cell proliferation and their dysfunction resulting from glomerulonephritis contribute to extracellular matrix degradation and remodeling and ultimately to glomerular fibrosis. Mesangial expansion and the degree of fibrosis present correlate inversely with glomerular filtration rate. It is a critical step in the progression to end-stage renal disease.

#### Signaling

TGF $\beta$ 1 and EGF activate their receptors (TGFBR2 and EGFR), and through the DAXX regulatory protein and EGFR/PI3K/PKC, signaling pathways mediate the MAPK cascade leading to the activation of the transcriptional factors JUN and FOS. JUN and FOS induce cell proliferation and the production of matrix metalloproteinases (MMP2, MMP3, MMP9, and MMP12) and fibronectin (FN1), which participate in the degradation of extracellular matrix and the progression of fibrosis. The subsequent transphosphorylation of TGFBR1 by TGFBR2 activates TGFBR1 and allows it to interact with SMAD2, SMAD3, and SMAD4 and form a heterotetrameric complex, which then translocates into the nucleus. Within the nucleus, that complex cooperates with the transcription factor SP1. Activated SP1 promotes the expression of collagen types I and IV that are involved in the development of fibrosis. The growth factors PDGFB, PDGFD, and FGF2 bind to their respective receptors PDGFRB and FGFR1 and in turn activate JAK2/STAT3/MYC signaling, which leads to mesangial cell proliferation (Liu, 2006; Masani et al., 2014; Schnaper et al., 2003).



**FIG. 6** Pathway 3: Mesangial cell dysfunction in glomerulonephritis.

## References

- Disease number #305800, #609814, #134370, #152700, and others in Online Mendelian Inheritance in Man (OMIM® database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code N05. Diseases of the genitourinary system (N00-N99). (ICD-10, <https://icdlist.com>). ICD-11: disease code GB4Z/GB40/GB4Y.
- Barbour, T.D., Pickering, M.C., Cook, H.T., 2013. Recent insights into C3 glomerulopathy. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* 28, 1685–1693. <https://doi.org/10.1093/ndt/gfs430>.
- Boor, P., Ostendorf, T., Floege, J., 2014. PDGF and the progression of renal disease. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* 29 (Suppl. 1), i45–i54. <https://doi.org/10.1093/ndt/gft273>.
- Djudjaj, S., Boor, P., 2018. Cellular and molecular mechanisms of kidney fibrosis. *Mol. Aspects Med.* <https://doi.org/10.1016/j.mam.2018.06.002>.
- Fearn, A., Sheerin, N.S., 2015. Complement activation in progressive renal disease. *World J. Nephrol.* 4, 31–40. <https://doi.org/10.5527/wjn.v4.i1.31>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Kronbichler, A., Leirer, J., Oh, J., Meijers, B., Shin, J.I., 2016. Immunologic changes implicated in the pathogenesis of focal segmental glomerulosclerosis. *BioMed Res. Int.* 2016, 2150451. <https://doi.org/10.1155/2016/2150451>.
- Lee, H.S., 2012. Mechanisms and consequences of TGF- $\beta$  overexpression by podocytes in progressive podocyte disease. *Cell Tissue Res.* 347, 129–140. <https://doi.org/10.1007/s00441-011-1169-7>.
- Liu, Y., 2006. Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int.* 69, 213–217. <https://doi.org/10.1038/sj.ki.5000054>.
- Loeffler, I., Wolf, G., 2014. Transforming growth factor- $\beta$  and the progression of renal disease. *Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc. Eur. Ren. Assoc.* 29 (Suppl. 1), i37–i45. <https://doi.org/10.1093/ndt/gft267>.
- Masani, N., Jhaveri, K.D., Fishbane, S., 2014. Update on membranoproliferative GN. *Clin. J. Am. Soc. Nephrol.* 9, 600–608. <https://doi.org/10.2215/CJN.06410613>.
- Schnaper, H.W., Hayashida, T., Hubchak, S.C., Poncelet, A.-C., 2003. TGF-beta signal transduction and mesangial cell fibrogenesis. *Am. J. Physiol. Renal Physiol.* 284, F243–F252. <https://doi.org/10.1152/ajprenal.00300.2002>.
- Shankland, S.J., 2006. The podocyte's response to injury: role in proteinuria and glomerulosclerosis. *Kidney Int.* 69, 2131–2147. <https://doi.org/10.1038/sj.ki.5000410>.
- Wiggins, R.C., 2007. The spectrum of podocytopathies: a unifying view of glomerular diseases. *Kidney Int.* 71, 1205–1214. <https://doi.org/10.1038/sj.ki.5002222>.
- Zipfel, P.F., Skerka, C., Chen, Q., Wiech, T., Goodship, T., Johnson, S., Fremeaux-Bacchi, V., Nester, C., de Córdoba, S.R., Noris, M., Pickering, M., Smith, R., 2015. The role of complement in C3 glomerulopathy. *Mol. Immunol.* 67, 21–30. <https://doi.org/10.1016/j.molimm.2015.03.012>.

## CHAPTER

## 13.3

## Polycystic kidney disease

Polycystic kidney disease (PKD) in 90% of cases is inherited from parents, while approximately 10% of cases are the result of a spontaneous gene mutation. Polycystic kidney disease the most common inherited kidney disease may associate with multiple gastrointestinal and cardiovascular abnormalities ([Ferri and Ferri, 2018](#)).

Polycystic kidney disease is a systemic inherited disorder leading to cysts formation and growth in multiple organs including kidneys (mainly), liver, and pancreas. ([Ferri and Ferri, 2018](#)).

An enormous number of mutations in the PKD1 and PKD2 and PKHD1 genes cause polycystic kidney disease. Mutations in either the PKD1 or PKD2 genes can evoke autosomal dominant polycystic kidney disease (ADPKD) that is the most common form of this disease and the most frequent monogenic disorder. Patients with PKD1 mutations exhibit a faster disease progression that often begins to manifest in those approximately 50 years old. Mutations in the PKHD1 gene cause a rare autosomal recessive polycystic kidney disease (ARPKD) in which cyst formation initiates in utero leading to underdeveloped kidneys and resulting in newborn deaths. Most mutations in the PKD1 and PKD2 and PKHD1 genes result in a nonfunctional version of the polycystins1,2 (PKD1 and PKD2) and polyductin (fibrocystin, PKHD1) proteins, or they disrupt the interactions of the protein and modify the molecular signaling pathways within the cell (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

Polycystic kidney disease symptoms may include pain in the abdomen and back, high blood pressure, dysuria (urination disorder), and hematuria (blood in the urine). Polycystic kidney disease can cause chronic renal failure, and there is currently no effective treatment for PKD.

The molecular mechanisms underlying polycystic kidney disease development are not entirely understood. The pathogenesis of this disease results from cyst formation in the renal tubules and the subsequent tubule dysfunction. The cysts are probably formed as dilations in the renal tubules as a result of increased cellular proliferation and growth.

An abnormal epithelial cell phenotype and extracellular matrix alterations result in spherical but not tubular structures. Then the dilations are stretched by fluid and expand through increased cell proliferation. They become isolated from the parental tubule and form the numerous cysts, which lead to structurally anomalous and nonfunctioning renal tubules along with massive renal enlargement (Finnigan and Leslie, 2018; Ghata and Cowley, 2017). Disruption of the activity of the polycystins on renal epithelial cell membrane stimulates cell proliferation, growth, and the fluid secretion that cause cyst formation.

**Pathway 1.** *Formation of cysts in polycystic kidney disease (Fig. 7).*

Polycystic kidney disease results from ciliopathies. The polycystins and fibrocystin are found on the apical membrane of renal tubule epithelial cells, which are located in primary cilia and then spread into the tubule lumen. Presumably the cilia act as sensors. When they are flexed by urine flow, they translate that fluid flow into chemical signals that regulate tubular development and function.

The loss of cilia alone is inadequate to cause cyst formation. However, a large body of evidence connects mutations in the genes encoding the polycystins and fibrocystin with the origin of cyst formation.

**Pathway 2.** *Dysfunction of cilia in polycystic kidney disease (Fig. 8).*

## Key cellular contributors and processes

### Cilia

#### Anatomic structure

Cilia are thin protuberances (less than 1 μm in width and from 3 to 2 mm in length) on the surface of eukaryotic cells that contain microtubule cytoskeleton structures. Cilia can be multiple or single and motile or non-motile (primary). The motile cilia are responsible for cell locomotion or movement of fluids surrounding the cell, whereas primary cilia serve as receptor organelles. Cilia are essential for the development and functioning of certain animal tissues.

### Ciliopathy

#### Disease

Ciliopathies are group of genetic disorders with a wide spectrum of phenotypes caused by mutations in genes encoding ciliary proteins that affect cilia structure or function.

### Cyst

#### Anatomic structure

A cyst is a pathological closed cavity in a tissue. Cysts have a distinct wall and may contain air, fluids, or semisolid material. Cells forming the wall of a cyst are abnormal compared with the surrounding cells in the nearby tissue.

### Intraflagellar transport

#### Process

Intraflagellar transport is a specialized intracellular process in eukaryotes essential for the biogenesis of cilia. It is a bidirectional transport of structural and functional ciliary components along microtubules to the tip of the cilium and back to the cell body.

## Pathway 1

### Formation cysts in polycystic kidney disease (Fig. 7)

#### Incoming signals

Polycystins1,2 are polyfunctional transmembrane proteins expressed on the basolateral plasma membrane and in the primary cilia located on the apical surface of renal tubule epithelial cells. The functions of PKD1 and PKD2 are not fully understood. These proteins can work together to promote healthy kidney development and kidney organization, while mutant PKD1 and PKD2 are associated with the formation of cysts.

#### Outcome effects

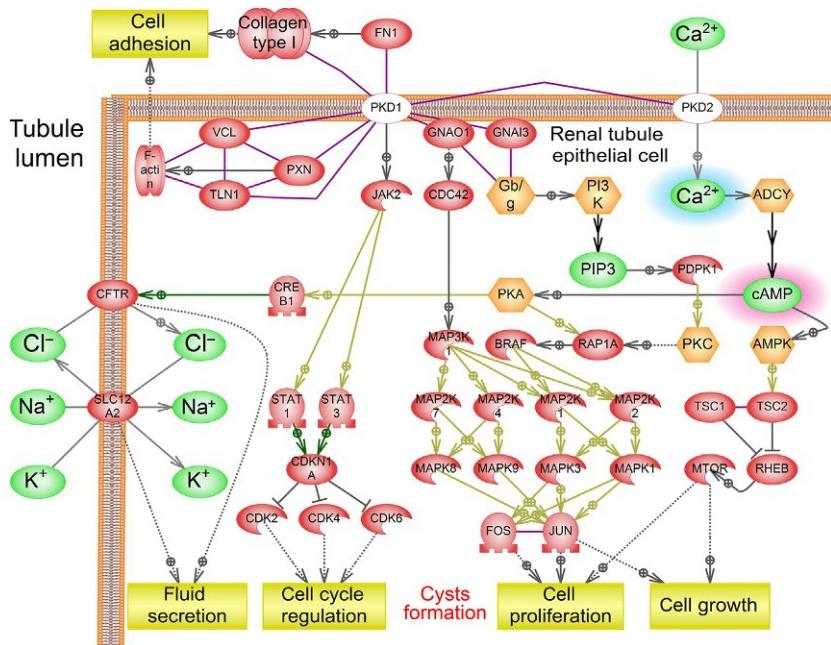
Cyst development and their permanent extension lead to progressive kidney enlargement, the transformation of their architecture, fibrosis, and the loss of kidney function.

#### Signaling

PKD2 is a nonselective ion channel that is permeable to  $\text{Ca}^{2+}$  and other cations. Intracellular calcium participates in the regulation of cyclic adenosine monophosphate (cAMP) levels. Low cytosolic calcium concentrations cannot inhibit the adenylate cyclase (ADCY) enzyme that catalyzes cAMP synthesis. Increased cAMP levels promote epithelial cell proliferation and growth by activating the PKA/MAPKs and AMPK/mTOR signalings. Activated PKA phosphorylates the transcription factor CREB1, which in turn promotes expression of the CFTR gene encoding the cystic fibrosis transmembrane regulator. The CFTR protein transports chloride ions into the cyst and triggers the increased transepithelial activity of ion transporters on the apical membrane, such as SLC12A2, that provoke abnormal fluid secretion into the cysts.

PKD1 is a large multidomain protein. The PKD1 extracellular domains are implicated in cell-cell and cell-matrix interactions, the cytoplasmic PKD1 tail interacts with many proteins that participate in various cellular processes, and mutations in different PKD1 domains lead to a diverse array of abnormal cellular function. PKD1 activates the transcription factors STAT1 and STAT3 by Janus kinase 2 (JAK2) phosphorylation leading to inhibition of the cyclin-dependent kinases 2, 4, and 6 (CDK2, CDK4, and CDK6) that regulate cell cycle progression. PKD1 can function as a G protein-coupled receptor and bind the G proteins GNAI3 and GNAO1, which in turn triggers PI3K/PKC/MAPK and CDC42/MAPK signalings. These pathways activate the JUN and FOS transcription factors that

control cellular proliferation and growth. Under normal conditions, PKD2 can repress PKD1-mediated G-protein signaling. PKD1 also interacts (not shown) with a negative regulator of mTOR activity termed tuberin (TSC2). The PKD1 extracellular domains bind to and form a complex with extracellular matrix proteins such as fibronectin 1 (FN1) and collagen type I. They also bind to intracellular cytoskeletal proteins such as paxillin (Pxn), talin 1 (TLN), and vinculin (VCL). These proteins and their interactions are involved with cell adhesion. Disturbance of cell-cell and cell-matrix junctions may be important for cystogenesis ([Chapin et al., 2010](#); [Merrick et al., 2014](#); [Paul and Vanden Heuvel, 2014](#); [Terryn et al., 2011](#)).



**FIG. 7** Pathway 1: Formation of cysts in polycystic kidney disease.

## Pathway 2

### Dysfunction of cilia in polycystic kidney disease ([Fig. 8](#))

#### Incoming signals

The pathology of polycystic kidney disease is linked to primary cilia disorganization and/or to their improper functioning as sensory antennae of fluid flow in the kidney. These structural and functional abnormalities have been shown to be associated with mutations in the PKD1, PKD2, and PKHD1 genes.

#### Outcome effects

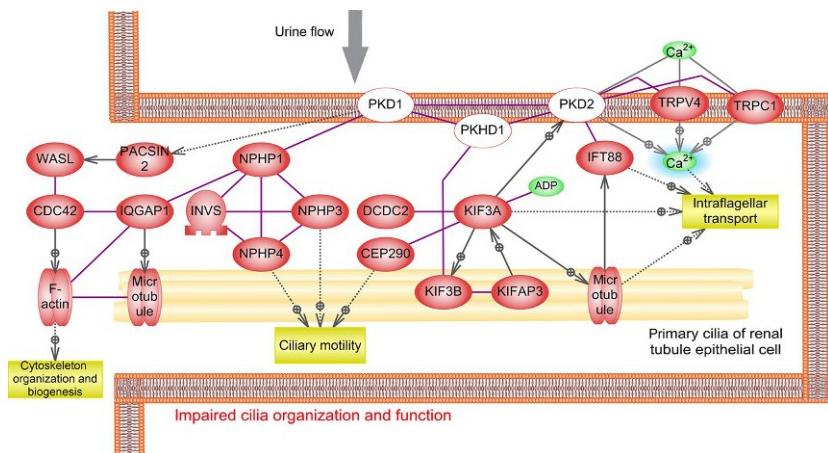
Impaired cilia activity on the renal tubule epithelial cell surface along with cytoskeleton disorganization may contribute to the cyst development and renal tubular dysfunction characteristic of this disease.

#### Signaling

In the kidney a mechanical response is evoked by cilium bending that triggers the calcium influx necessary for many cellular processes. The genesis of cilia and their proper function depend on the intraflagellar transport for which calcium ions are also needed. The PKD1 large extracellular domain is thought to be a mechanosensor that detects urine flow. When PKD1 is activated, it interacts with PKD2, PKHD1, and transient cation channels (TRPV4 or TRPC1) to promote calcium entry into the cell.

Intraflagellar transport is an essential process for ciliogenesis. Anterograde intraflagellar transport is performed by the heterotrimeric kinesin-II motor protein complex along the microtubules formed by kinesin 3A (KIF3A), kinesin 3B (KIF3B), and the kinesin-associated protein 3 (KIFAP3). KIF3A and KIF3B interact with PKD2 and PKHD1. While the role of this complex is not clear, PKHD1 can stimulate PKD2 channel activity in the presence of KIF3B. The centrosomal protein CEP290 and the doublecortin domain-containing protein 2 (DCDC2) interact with KIF3A to regulate the ciliary motility necessary for cilia mechanosensory activity.

In polycystic kidney disease the cellular cytoskeleton and intercellular contacts become disorganized and together contribute to cyst formation. The ciliary axoneme (i.e., the inner rod of a cilium) connects with the cytoskeleton. Moreover, polycystins are part of the extracellular matrix, which mediate cell interactions through multiprotein complexes. PKD1 can regulate cytoskeleton organization via pasin 2 (PASCIN2) and the nephrocystins (NPHP1, NPHP3, and NPHP4). Mutations in PKD1 and PKD2 or PKHD1 may disturb the fluid-flow sensing mechanism or the structure of cilia, thus inhibiting cilia motility and their corresponding functions ([Dell, 2015; Fedeles et al., 2014; Ferreira et al., 2015; Retailleau and Duprat, 2014; Wang and Dong, 2013; Yao et al., 2014](#)).



**FIG. 8** Dysfunction of cilia in polycystic kidney disease.

## References

- Disease number #173900, #613095, #263200, and others in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code Q61.11-Q61.3. Congenital malformations, deformations and chromosomal abnormalities (Q00-Q99). (ICD-10, <https://icdlist.com>). ICD-11: disease code GB8Z/GB80/GB81.
- Chapin, H.C., Rajendran, V., Caplan, M.J., 2010. Polycystin-1 surface localization is stimulated by polycystin-2 and cleavage at the G protein-coupled receptor proteolytic site. *Mol. Biol. Cell* 21, 4338–4348. <https://doi.org/10.1091/mbc.E10-05-0407>.
- Dell, K.M., 2015. The role of cilia in the pathogenesis of cystic kidney disease. *Curr. Opin. Pediatr.* 27, 212–218. <https://doi.org/10.1097/MOP.00000000000000187>.
- Fedeles, S.V., Gallagher, A.-R., Somlo, S., 2014. Polycystin-1: a master regulator of intersecting cystic pathways. *Trends Mol. Med.* 20, 251–260. <https://doi.org/10.1016/j.molmed.2014.01.004>.
- Ferreira, F.M., Watanabe, E.H., Onuchic, L.F., 2015. Polycystins and molecular basis of autosomal dominant polycystic kidney disease. In: Li, X. (Ed.), *Polycystic Kidney Disease*. Codon Publications, Brisbane.
- FERRI, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Finnigan, N.A., Leslie, S.W., 2018. Polycystic kidney disease, adult. In: StatPearls. StatPearls Publishing, Treasure Island, FL.
- Ghata, J., Cowley, B.D., 2017. Polycystic kidney disease. *Compr. Physiol.* 7, 945–975. <https://doi.org/10.1002/cphy.c160018>.
- Merrick, D., Bertuccio, C.A., Chapin, H.C., Lal, M., Chauvet, V., Caplan, M.J., 2014. Polycystin-1 cleavage and the regulation of transcriptional pathways. *Pediatr. Nephrol.* 29, 505–511. <https://doi.org/10.1007/s00467-013-2548-y>.
- Paul, B.M., Vanden Heuvel, G.B., 2014. Kidney—polycystic kidney disease. *Wiley Interdiscip. Rev. Dev. Biol.* 3, 465–487. <https://doi.org/10.1002/wdev.152>.
- Retailleau, K., Duprat, F., 2014. Polycystins and partners: proposed role in mechanosensitivity. *J. Physiol.* 592, 2453–2471. <https://doi.org/10.1113/jphysiol.2014.271346>.
- Terry, S., Ho, A., Beauwens, R., Devuyst, O., 2011. Fluid transport and cystogenesis in autosomal dominant polycystic kidney disease. *Biochim. Biophys. Acta* 1812, 1314–1321. <https://doi.org/10.1016/j.bbadic.2011.01.011>.
- Wang, S., Dong, Z., 2013. Primary cilia and kidney injury: current research status and future perspectives. *Am. J. Physiol. Renal Physiol.* 305, F1085–F1098. <https://doi.org/10.1152/ajprenal.00399.2013>.
- Yao, G., Su, X., Nguyen, V., Roberts, K., Li, X., Takakura, A., Plomann, M., Zhou, J., 2014. Polycystin-1 regulates actin cytoskeleton organization and directional cell migration through a novel PC1-Pacsin 2-N-Wasp complex. *Hum. Mol. Genet.* 23, 2769–2779. <https://doi.org/10.1093/hmg/ddt672>.

## CHAPTER

## 13.4

## Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting up to 25% of reproductive-age women. Patients with PCOS have cysts in their ovaries and higher than normal levels of male hormones. This hormone imbalance undermines the ability of women to get pregnant. PCOS is the most common cause of anovulatory infertility ([Ferri and Ferri, 2018](#)).

Polycystic ovary syndrome (PCOS) is characterized by an accumulation of incompletely developed follicles in the ovaries due to anovulation and associated with ovarian androgen production. ([Ferri and Ferri, 2018](#)).

PCOS symptoms include irregular menstrual periods, pelvic pain, excess body and facial hair, acne, obesity, increased anxiety, and depression. The symptoms usually start in early adulthood. PCOS is associated with reproductive, cosmetic, and psychological problems, and later in life, women with PCOS have an increased risk of developing insulin-resistant type 2 diabetes mellitus, hypertension, atherosclerosis, and endometrial and ovarian cancers.

PCOS is a multifactorial disorder that results from a bouquet of endocrine, genetic, metabolic, and lifestyle causes, some of which have not been fully established. Although a family history of disease suggests a heritable aspect in this syndrome, most of the susceptibility genes remain to be identified. It is known that genetic variants, which lead to an elevated level of androgens, luteinizing hormone, and anti-Mullerian hormone along with decreased levels of follicle-stimulating hormone and sex hormone-binding globulin, increase the risk of developing PCOS. Other genetic changes associated with PCOS occur in genes that code for the proteins involved with insulin and cholesterol synthesis and regulation of their respective levels (Genetics Home Reference, <https://ghr.nlm.nih.gov>).

The molecular pathophysiology of PCOS has not yet been fully elucidated. Within a healthy ovary during the normal menstrual cycle, follicles grow until a mature follicle ruptures to release an egg that then enters the fallopian tube (ovulation). Other, nonegg producing follicles

dissolve. This process occurs in the middle of the cycle and is regulated by a coordinated network of many hormones including gonadotropic hormones, female sex hormones (estrogens), and male sex hormones (androgens). In PCOS the processes of egg maturation and ovulation are blocked, leading to the formation of cysts, follicles filled with fluid. The absence of ovulation and disturbances in the menstrual cycle prevents conception.

PCOS is a heterogeneous condition with various phenotypes. Hyperandrogenism and insulin resistance are thought to be the two main features of the PCOS-related conditions. Hyperandrogenism can be a consequence of the disruption of pituitary and hypothalamus function, which in turn impairs the production of luteinizing and follicle-stimulating hormones.

**Pathway 1.** *Disruption of luteinizing hormone and follicle-stimulating hormone secretion in PCOS (Fig. 9).*

The gonadotropic hormones regulate the synthesis of both androgens and estrogens. About 60%–85% of women with PCOS have clinical hyperandrogenism in the form of excessive androgen biosynthesis in the ovaries.

**Pathway 2.** *Impaired steroidogenesis in PCOS (Fig. 10).*

## Key cellular contributors and processes

Hyperandrogenism

Pathological condition

Hyperandrogenism is a medical condition characterized by excessive levels of androgens in a female body.

Insulin resistance

Pathological condition

Insulin resistance is a pathological condition characterized by the impaired ability of insulin targeted tissues to respond to insulin.

Ovary cyst

Anatomic structure

An ovarian cyst is a fluid-filled closed cavity inside the ovary. Ovarian cysts are often asymptomatic; however, they may produce abdominal or back pain and disrupt the normal progression of the menstrual cycle.

Ovary follicle

Anatomic structure

The ovarian follicle is a spherical structure inside the ovaries that contains an egg (ovum, egg cell, or oocyte). Ovarian follicles also contain granulosa (or follicular) cells that surround the oocyte and theca cells that secrete hormones, which affect the progression of the menstrual cycle.

## Pathway 1

### Disruption of luteinizing hormone and follicle-stimulating hormone secretion in PCOS ([Fig. 9](#))

#### Incoming signals

PCOS develops as a result of an imbalance of the female sex hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Gonadotropin-releasing hormone (GNRH1, gonadoliberin) stimulates anterior pituitary gland (adenohypophysis) cells to secrete LH and FSH.

Hypothalamic neurons produce GNRH1 in a pulsatile pattern. High-frequency GNRH1 pulses stimulate LH beta-subunit (LHB) release, whereas low-frequency GNRH1 pulses are required for FSH beta-subunit (FSHB) production. Although LHB and FSHB are both secreted from the same gonadotropic cells in response to GNRH1 pulsations, serum concentrations of LHB and FSHB change throughout the menstrual cycle.

In the course of PCOS, enhanced high-frequency GNRH1 pulsations induce increased LHB release. The etiology of abnormal GNRH1 pulsations in PCOS remains unclear; however, it is assumed that the hypothalamic-pituitary-gonadal axis is hyperactive due to a decreased sensitivity of GNRH pulse generation and impaired negative gonadal hormone feedback.

The regulatory proteins activin, inhibin, and follistatin (FST) can stimulate or inhibit FSH secretion in certain phases of the menstrual cycle. In PCOS the serum levels of inhibitory FST are frequently increased.

#### Outcome effects

Increased levels of LHB facilitate androgen synthesis, whereas decreased FSHB levels result in an arrest of ovarian follicle development.

#### Signaling

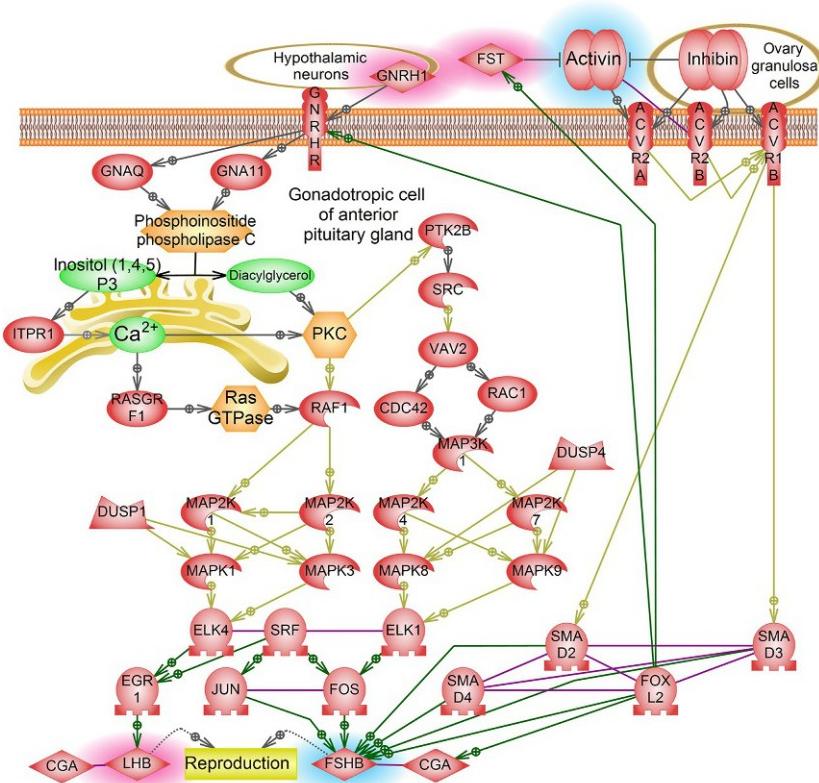
The mechanisms of differential secretion of LHB and FSHB have yet to be completely understood. In gonadotropic cells of the anterior pituitary gland, GNRH1 acts on its receptor GNRHR to transmit the signal to phospholipase C via the G proteins GNA11 and GNAQ. However, it is not entirely clear which G proteins are involved in the signaling that preferentially induces either LHB or FSHB secretion. Activated by G proteins, phospholipase C mediates production of diacylglycerol and inositol, which in turn bind the inositol 1,4,5-triphosphate receptor (ITPR1) on the endoplasmic reticulum. ITPR1 causes an increase in the cytosolic  $\text{Ca}^{2+}$

concentration and the activation of MAPK signaling cascades. GNRH1 stimulates MAPK1,3 through the PKC/RAF1 signaling pathway and MAPK8,9 through PKC/SRC/CDC42 signaling pathway.

Different patterns of MAPK activation in response to various GNRH1 pulse frequencies suggest that they may be responsible for the differential secretion of LHB and FSHB. MAPK1,3 activate the transcription factor EGR1, thus promoting LHB expression, and MAPK8,9 activate the transcription factors FOS and JUN, which are involved in FSHB promotion.

Activins are gonadal peptides that also stimulate FSH production. They bind to type 2 receptors (ACVR2A and ACVR2B) and induce the recruitment and activation of the type 1 receptor (ACVR1B), which consequently phosphorylates SMAD2 and SMAD3. Activated SMADs translocate to the nucleus and bind SMAD4 to form a complex that regulates the expression of target genes.

Inhibin is an antagonist of activin. FSH stimulates inhibin production from ovarian granulosa cells. Inhibin, in turn, downregulates FSH secretion. The exact mechanism of inhibin action has yet to be discovered. Supposedly, inhibin competitively binds activin receptors, thereby blocking the subsequent binding of activating. Follistatin (FST) is another gonadal peptide that binds activin and prevents its interaction with activating receptors (Das and Kumar, 2018; McCartney et al., 2002; Moore and Campbell, 2017; Thompson and Kaiser, 2014).



**FIG. 9** Pathway 1: Disruption of luteinizing hormone and follicle-stimulating hormone secretion in PCOS.

## Pathway 2

### Impaired steroidogenesis in PCOS (Fig. 10)

#### Incoming signals

In PCOS, serum LHB levels are typically increased, and FSHB levels are often decreased. Disturbances of the FSHB to LHB ratio is one of the major causes of PCOS. Luteinizing hormone (LHB) hypersecretion leads to enhanced androgen production by the ovarian theca cells. Reduced FSHB secretion causes a deficit of enzymes that synthesize estrogens in ovarian granulosa cells.

Insulin (INS) enhances LHB action by triggering the expression of steroidogenic enzymes. Hyperinsulinemia diminishes granulosa cell proliferation and subsequent follicle growth by enhancing the activity of serum insulin-like growth factors (IGF1 and IGF2) through the suppression of insulin-like growth factor-binding protein (IGFBP1) production in the liver.

PCOS is also associated with the development of insulin resistance (read more about insulin resistance in type 2 diabetes mellitus). Insulin resistance leads to compensatory hyperinsulinemia with its complex effects on the regulation of both metabolism and hormone production.

#### Outcome effects

Excess androgen production and estrogen insufficiency in certain phases of the menstrual cycle along with hyperinsulinemia contribute to the arrest of follicle maturation and their cystic degeneration, the block of ovulation, and the disruption of menstrual cycle progression seen in PCOS.

Increased androgen synthesis also acts peripherally in other androgen-sensitive tissues. For example, when stimulated by hyperinsulinemia, steroid-5-alpha-reductase stimulates a high rate of testosterone to dihydrotestosterone conversion that leads to hirsutism, acne, and other PCOS manifestations of the skin (read more about steroid-5-alpha-reductase in Acne Vulgaris) ([De Leo et al., 2016](#); [Fenichel et al., 2017](#); [Meier, 2018](#); [Mykhalchenko et al., 2017](#); [Patel, 2018](#); [Teede et al., 2010](#)).

#### Signaling

In theca cells of the ovary, LH acts on the luteinizing hormone receptor (LHCGR), which in turn transmits the signal intracellularly to adenylate cyclase (ADCY) via the regulatory protein GNAS. Then, ADCY catalyzes the synthesis of the second messenger cAMP. Further, increased cAMP levels activate protein kinase A (PKA), thereby stimulating the activity of

the steroidogenic acute regulatory protein (STAR) that transports cholesterol into the mitochondria.

Cholesterol is a primary precursor in steroid hormone synthesis. Cytochrome P450 11A1 (CYP11A1) converts cholesterol to pregnenolone, and CYP17A1 converts pregnenolone to 17-hydroxypregnenolone and further into dehydroepiandrosterone. Steroid dehydrogenase HSD3B2 catalyzes the conversion of dehydroepiandrosterone to androstenedione, and HSD17B1 transforms androstenedione to testosterone. In the granulosa cells of the ovary, cytochrome P450 19A1 (CYP19A1, aromatase) converts androstenedione and testosterone to estrogens (estrone and estradiol).

The increased levels of LHB cause hyperstimulation or overexpression of androgen biosynthesis. The aromatase activity deficiency in granulosa cells, reported in many PCOS patients, can lead to excess androgen levels and abnormal ovarian follicle development.

Insulin signaling is involved in the control of enzyme expression in androgen biosynthesis. In theca cells, insulin (INS) acts via IRS and IGF1R to stimulate PI3K leading to the activation of the transcription factor NR5A1 that promotes increased expression of STAR, CYP11A1, and CYP17A1. Moreover, insulin decreases the production of globulin (SHBG) in the liver. SHBG is a regulatory glycoprotein responsible for the transport of androgens and estrogens.

Also, in the granulosa cells of the ovary, FSHB binds follicle-stimulating hormone receptor (FSHR) and activates the ADCY/MAPK pathway. MAPKs induce the transcription factors CREB and GATA4, thus promoting expression of CYP19A1. CYP19A1 levels are downregulated since FSHB levels are low in PCOS.

FSHB, together with insulin-like growth factors (IGF1 and IGF2), activates the PI3K/AKT1 pathway, which in turn leads to the inhibition of FOXO1, a negative regulator of granulosa cell proliferation necessary for correct follicle development. However, decreased FSHB levels and increased IGF levels observed in PCOS impede this regulation ([Calogero et al., 2011](#); [Crespo et al., 2018](#); [Macut et al., 2017](#); [Rosenfield and Ehrmann, 2016](#); [Yau et al., 2017](#)).

## II. Human disease pathways

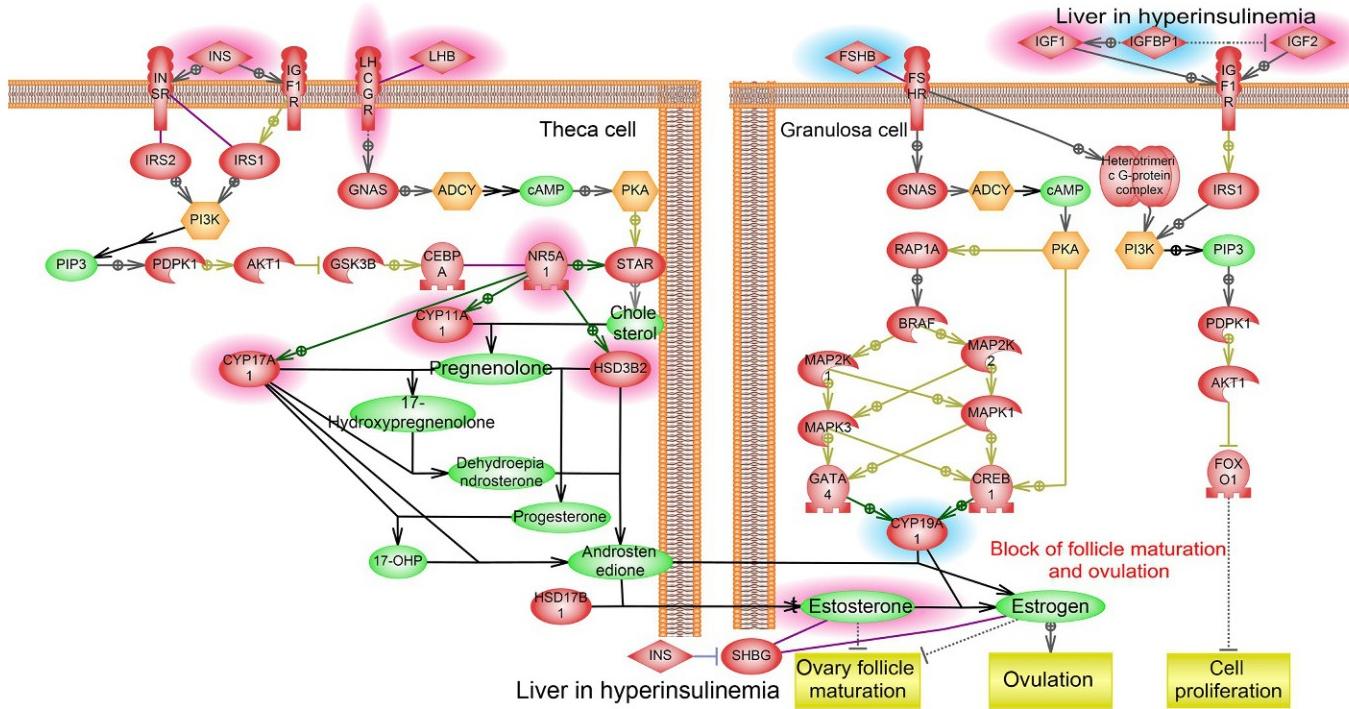


FIG. 10 Pathway 2: Impaired steroidogenesis in PCOS.

## References

- Disease number #184700 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code E28.2. N83.2 Other and unspecified ovarian cysts. (ICD-10, <https://icdlist.com>). ICD-11: disease code 5A80.1.
- Calogero, A.E., Calabrò, V., Catanuso, M., Condorelli, R.A., La Vignera, S., 2011. Understanding polycystic ovarian syndrome pathogenesis: an updated of its genetic aspects. *J. Endocrinol. Investig.* 34, 630–644. <https://doi.org/10.3275/7746>.
- Crespo, R.P., Bachega, T.A.S.S., Mendonça, B.B., Gomes, L.G., 2018. An update of genetic basis of PCOS pathogenesis. *Arch. Endocrinol. Metab.* 62, 352–361. <https://doi.org/10.20945/2359-3997000000049>.
- Das, N., Kumar, T.R., 2018. Molecular regulation of follicle-stimulating hormone synthesis, secretion and action. *J. Mol. Endocrinol.* 60, R131–R155. <https://doi.org/10.1530/JME-17-0308>.
- De Leo, V., Musacchio, M.C., Cappelli, V., Massaro, M.G., Morgante, G., Petraglia, F., 2016. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod. Biol. Endocrinol.* 14, 38. <https://doi.org/10.1186/s12958-016-0173-x>.
- Fenichel, P., Rougier, C., Hieronimus, S., Chevalier, N., 2017. Which origin for polycystic ovaries syndrome: genetic, environmental or both? *Ann. Endocrinol.* 78, 176–185. <https://doi.org/10.1016/j.ando.2017.04.024>.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Macut, D., Bjekić-Macut, J., Rahelić, D., Doknić, M., 2017. Insulin and the polycystic ovary syndrome. *Diabetes Res. Clin. Pract.* 130, 163–170. <https://doi.org/10.1016/j.diabres.2017.06.011>.
- McCartney, C.R., Eagleson, C.A., Marshall, J.C., 2002. Regulation of gonadotropin secretion: implications for polycystic ovary syndrome. *Semin. Reprod. Med.* 20, 317–326. <https://doi.org/10.1055/s-2002-36706>.
- Meier, R.K., 2018. Polycystic ovary syndrome. *Nurs. Clin. North Am.* 53, 407–420. <https://doi.org/10.1016/j.cnur.2018.04.008>.
- Moore, A.M., Campbell, R.E., 2017. Polycystic ovary syndrome: understanding the role of the brain. *Front. Neuroendocrinol.* 46, 1–14. <https://doi.org/10.1016/j.yfrne.2017.05.002>.
- Mykhalchenko, K., Lizneva, D., Trofimova, T., Walker, W., Suturina, L., Diamond, M.P., Azziz, R., 2017. Genetics of polycystic ovary syndrome. *Expert. Rev. Mol. Diagn.* 17, 723–733. <https://doi.org/10.1080/14737159.2017.1340833>.
- Patel, S., 2018. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. *J. Steroid Biochem. Mol. Biol.* 182, 27–36. <https://doi.org/10.1016/j.jsbmb.2018.04.008>.
- Rosenfield, R.L., Ehrmann, D.A., 2016. The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr. Rev.* 37, 467–520. <https://doi.org/10.1210/er.2015-1104>.
- Teede, H., Deeks, A., Moran, L., 2010. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med.* 8, 41. <https://doi.org/10.1186/1741-7015-8-41>.
- Thompson, I.R., Kaiser, U.B., 2014. GnRH pulse frequency-dependent differential regulation of LH and FSH gene expression. *Mol. Cell. Endocrinol.* 385, 28–35. <https://doi.org/10.1016/j.mce.2013.09.012>.
- Yau, T.T., Ng, N.Y., Cheung, L.P., Ma, R.C., 2017. Polycystic ovary syndrome: a common reproductive syndrome with long-term metabolic consequences. *Hong Kong Med. J. Xianggang Yi Xue Za Zhi* 23, 622–634. <https://doi.org/10.12809/hkmj176308>.

## CHAPTER

## 13.5

## Endometriosis

Endometriosis is an estrogen-dependent gynecological disease characterized by endometrial-like tissue growing outside of the uterine cavity, typically on the pelvic peritoneum, in the ovaries, and in the rectovaginal septum.

Endometriosis is defined as the presence of functioning endometrial glands and stroma outside the uterine cavity. (*Ferri and Ferri, 2018*).

Endometriosis typically results in extensive pelvic adhesions that often lead to pain, menorrhagia, and infertility. Endometriosis affects 10% of reproductive-age women (*Bulletti et al., 2010*).

Endometriosis is a heterogeneous disease that has phenotypically different endopelvic and extrapelvic manifestations. The pathogenic mechanisms underlying the disease are complex. Probably the initiation of the disease results from the process of retrograde menstruation. Though retrograde menstruation occurs in 76%–90% of reproductive-age women, only 10% of women develop endometriosis (*Ahn et al., 2017*).

Retrograde menstruation with direct implantation theory (Sampson's theory) coexists with other hypotheses of the origin of endometriosis (e.g., transformation of multipotential cells of the coelomic epithelium and transport of endometrial cells to distant sites by the uterine vascular and lymphatic systems and through autoimmune mechanisms) (*Ferri and Ferri, 2018*).

The pathogenesis of endometriosis may be represented in the following ways: (1) appearance of endometrial fragments outside of the uterus, (2) implantation and proliferation of ectopic endometrial cells (or endometriotic cells) with the formation of endometrioid heterotopies (or loci of ectopic endometrium), and (3) pathological manifestations (e.g., angiogenesis, inflammation, pain, and bleeding).

In ectopic endometrium, there are cyclic changes similar to the processes in the uterine endometrium, but these processes are not always synchronous, and endometrioid bleeding and pelvic pain are not always associated with menstruation. Persistent pain significantly reduces the patient's quality of life.

The progression of endometriosis is estrogen dependent and involves immune dysfunction and inflammation. Inflammatory mediators are thought to be able to stimulate the epigenetic transformation of menstrual endometrial fragments into the abnormal ectopic endometrium (Ahn et al., 2017). Genetic and epigenetic markers for endometriosis could be useful diagnostic tools, but to date the search for markers and polymorphisms associated with the disease is incomplete.

The exact mechanisms of disease progression are not clear. Several factors play a role in the development of the endometriosis. Estrogens drive endometriosis lesion development. However, there is also the connection between inflammation and expansion of the disease:

**Pathway 1.** Local estrogen production stimulates ectopic endometrium proliferation (Fig. 11).

Along with the progression of the disease, the development of progesterone resistance becomes another trigger factor.

**Pathway 2.** Progesterone resistance stimulates endometriotic cell survival (Fig. 12).

Enhanced angiogenesis is the second crucial factor for the development of endometriosis.

**Pathway 3.** Enhanced angiogenesis in endometriosis (Fig. 13).

## Key cellular contributors and processes

### Estradiol

Estradiol is the major steroid female sex hormone responsible for the maintenance of fertility.

### Progesterone

Progesterone is an essential steroid sex hormone involved in the regulation of the menstrual cycle, early pregnancy support, and embryogenesis.

### Retinoic acid

Retinoic acid is a vitamin A (retinol) derivative involved in the regulation of cell differentiation and embryonic development.

## Pathway 1

### Local estrogen production stimulates ectopic endometrium proliferation ([Fig. 11](#))

#### Incoming signals

Endometriotic cells proliferate in response to both systemic and locally produced estrogens. Estradiol is the dominant biologically active estrogen produced along with estrone and estriol in females. Ectopic endometriotic cells produce high levels of estradiol. Prostaglandin E2 (PGE2) is the effective activator of estrogen biosynthesis in ectopic endometrium, and it is an inducer of pain and inflammation in neighboring tissues. PGE2 production can be stimulated by estrogen, inflammatory mediators, and PGE2 itself with the last forming a positive feedback loop.

In endometriosis a high concentration of estradiol may be associated with locally increased aromatase (CYP19A1, a key enzyme of estrogen synthesis) activity in endometriotic cells. In contrast to normal endometrium, this elevated activity is not controlled by cyclical changes in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production.

Inflammation is a normal process in menstruation, but it became pathophysiological in endometriosis when menstrual fragments invade the peritoneal cavity. Chronic inflammation in endometriosis lesions stimulates the production of proinflammatory mediators such as cytokines or PGE2. PGE2 hyperproduction, in turn, supports the inflammatory cycle and impacts the development of pain.

#### Outcome effects

The influence of estrogens is not a necessary condition for the implantation of endometrial fragments. However, the presence of estrogens is required for the proliferation and growth of endometriotic cells.

In healthy endometrium the expression of ESR1 predominates over ESR2 although the balance varies throughout the menstrual cycle ([Huhtinen et al., 2012](#)). In ovarian endometriosis, ESR2 may be overexpressed, and therefore the ratio of ESR1/ESR2 decreases. Although the details remain indistinct, it is clear that estrogens drive endometriotic cell proliferation by regulating the ESR1/ESR2 ratio and by activating proliferative signaling cascades.

#### Signaling

Activated expression of PTGS2 in ectopic endometriotic cells, or in inflamed tissue, leads to the synthesis of the elevated levels of PGE2.

PGE2 acts on prostaglandin E receptors (PTGERs) to activate cAMP/PKA signaling. PKA activates nuclear transcription factors such as nuclear receptor subfamily 5 group A member 1 (NR5A1), CAMP responsive element-binding protein 1 (CREB1), CCAAT/enhancer-binding protein beta (CEPB), and possibly some others, which in turn initiate the transcription of the steroidogenic acute regulatory protein (STAR), CYP19A1, and other steroidogenic enzymes that play a role in estrogen synthesis.

STAR facilitates the first step of estrogen synthesis by transporting cholesterol from the cytosol into the mitochondria. Then, at least six different enzymes are involved with the conversion of cholesterol to estradiol. In humans, only one protein, CYP19A1, catalyzes the final conversion of precursor steroids into estrogens, so the expression of CYP19A1 has a substantial effect on estrogen biosynthesis and endometriosis progression.

Elevated expression levels of STAR and CYP19A1 have been demonstrated in the abnormal endometrium from women with endometriosis ([Huhtinen et al., 2012](#)). There is a hypothesis that the high levels of aromatase in endometriotic cells could be conditioned by downregulating its inhibitory transcription factors Wilms tumor 1 (WT1) and perhaps some others (not shown) ([Attar and Bulun, 2006](#)).

Several 17beta-hydroxysteroid dehydrogenases (HSD17B) proteins regulate the transformation between estradiol and estrone. HSD17B1 converts estrone to estradiol. HSD17B2 is thought to be the major enzyme responsible for inactivating the conversion estradiol to estrone in endometriosis. This assumes that expression of HSD17B2 in endometriosis may be low due to the development of progesterone resistance ([Huhtinen et al., 2012](#)). Also, estradiol can be inactivated via the formation of sulfate—or glucorone conjugates (e.g., by SULT1E1, sulfotransferase family 1E member 1). On the contrary, steroid sulfatase (STS) activity releases estrogens from their sulfate conjugates (i.e., estrone from estrone sulfate, estradiol from estradiol sulfate, and dehydroepiandrosterone from dehydroepiandrosterone sulfate) ([Bulun et al., 2010, 2012; Rizner, 2009; Sacco et al., 2012](#)).

## II. Human disease pathways

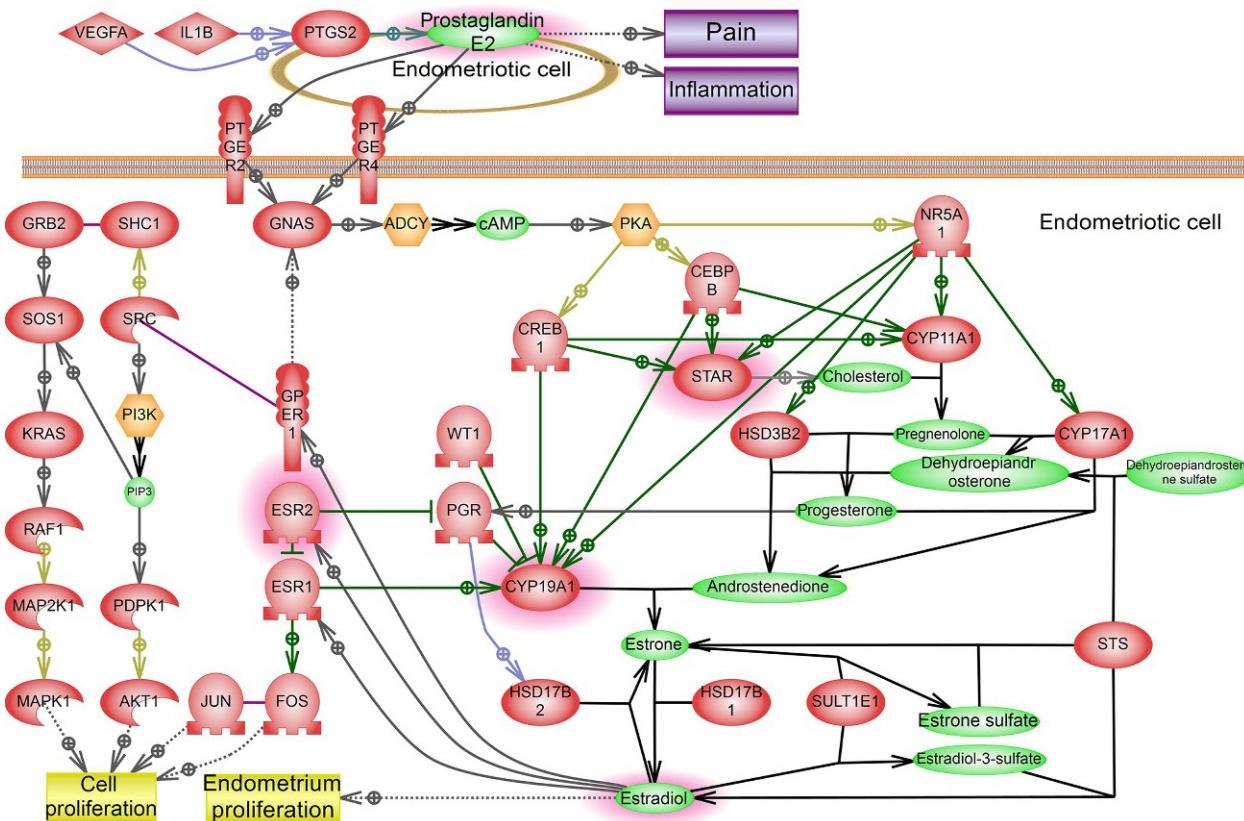


FIG. 11 Pathway 1: Local estrogen production stimulates ectopic endometrium proliferation.

## Pathway 2

### Progesterone resistance stimulates endometriotic cell survival (Fig. 12)

#### Incoming signals

Normally in the luteal phase of the menstrual cycle, progesterone induces endometrial decidualization and diminishes the effects of estrogen. Ectopic endometrium develops resistance to progesterone, one of the hallmarks of endometriosis. Progesterone resistance likely develops due to the downregulation of progesterone receptor (PGR) expression and related signaling mechanisms in endometrial cell. Regulation of PGR expression occurs because of increased methylation at the promoter and first exon of the *PGR* gene. The peritoneal fluid contains multiple factors such as hormones, cytokines, and growth factors that can stimulate cell surface receptors that lead to the activation of protein kinases that have been shown to suppress PGR activity via increased phosphorylation and subsequent degradation of the receptor via proteasome pathways (not shown) (McKinnon et al., 2018). Also, reduced retinoic acid uptake is reported in endometriosis and is linked to the decline of PGR activity. Retinoic acid is a vitamin A metabolite that normally has antiproliferative effects on the endometrium.

#### Outcome effects

Resistance to progesterone and the decline in retinoic acid synthesis or uptake inhibits physiological apoptosis and stimulates cell proliferation in ectopic endometrium.

#### Signaling

ESR2 may lead to the inhibition of PGR transcription in endometriosis. There is evidence that in the ovarian endometriosis, ESR2 is overexpressed and the ESR1/ESR2 ratio is decreased (Huhtinen et al., 2012). Chronic inflammation may provoke deficient methylation of the *ESR2* gene resulting in its overexpression.

PGR is necessary for the expression of the transcription factor forkhead box protein O1 (FOXO1), which is crucial for endometrium development because it inhibits estrogen production and cell proliferation.

Low PGR expression levels lead to decreased retinoic acid uptake in endometriosis most likely because of the greatly reduced expression of genes that regulate the cellular uptake of retinol (e.g., *STRA6*, stimulated

by retinoic acid 6) or progesterone responsiveness (e.g., *CYP26A1*, cytochrome P450 family 26 subfamily A member 1).

Normally, retinoic acid may promote either cell survival or apoptosis depending on the levels of its binding proteins including the apoptosis-inducing cellular RA-binding protein 2 (CRABP2) and the cell survival-promoting fatty acid-binding protein 5 (FABP5). CRABP2 directs retinoic acid toward retinoic acid receptor alpha (RARA), which results in growth arrest and apoptosis, whereas FABP5 directs retinoic acid to bind to peroxisome proliferator-activated receptor delta (PPARD), which promotes expression of prosurvival genes. The consequent decrease in the ratio of CRABP2 to FABP5 common in endometriosis may be important for guiding retinoic acid signaling pathways toward increasing the survival of endometriotic cells.

Retinoic acids also have been shown to mediate the regulation of HSD17B2, thus blocking estradiol degradation (i.e., inactivation) ([Ahn et al., 2017](#); [Aznaurova et al., 2014](#); [Bulun et al., 2010](#); [Macer and Taylor, 2012](#); [Pavone et al., 2010, 2011](#); [Pierzchalski et al., 2014](#); [Sokalska et al., 2013](#); [Wieser et al., 2012](#)).

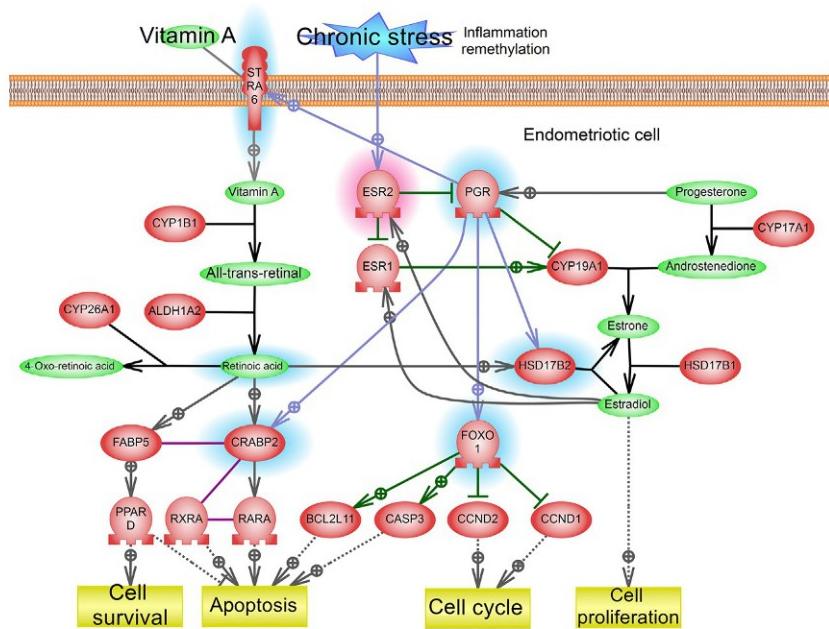


FIG. 12 Pathway 2: Progesterone resistance stimulates endometriotic cell survival.

## Pathway 3

### Enhanced angiogenesis in endometriosis (Fig. 13)

#### Incoming signals

Angiogenesis is a critical process in the establishment and growth of endometriotic lesions. The process of angiogenesis is stimulated by two central pathways that involve vascular endothelial growth factor A (VEGFA) and angiopoietin 1 (ANGPT1). Both VEGFA and ANGPT1 are strongly overexpressed in endometriotic lesions. It has been shown that the inhibition of VEGF leads to a significant decrease in endometriotic lesions.

#### Outcome effects

VEGFA and ANGPT1 act as endothelial cell (EC) mitogens, enhancing cell proliferation and migration and increasing the vascular permeability in ectopic endometrium. Vascularization is necessary for the formation and function of ectopic endometrium lesions.

#### Signaling

VEGFA and ANGPT1 are produced by macrophages and endometriotic cells. VEGFA binds to its main receptor VEGFR2 (KDR), and ANGPT1 binds TEK. KDR and TEK receptors initiate intracellular signaling cascades that facilitate actin cytoskeleton assembly and the expression of survival signals and the production of mediators of vasodilation. PI3K/AKT signaling activates the mTOR complex to increase levels of translation. The Src homology two domain-containing (SHC) transforming protein also passes the receptor signal and activates the universal RAF1 and RAS/MAPK pathways. Activated calcium-dependent signaling participates in nitric oxide (NO) and prostacyclin synthesis ([Djokovic and Calhaz-Jorge, 2014](#); [Girling and Rogers, 2009](#); [Hur et al., 2006](#); [Laschke and Menger, 2012](#); [Rocha et al., 2013](#)).

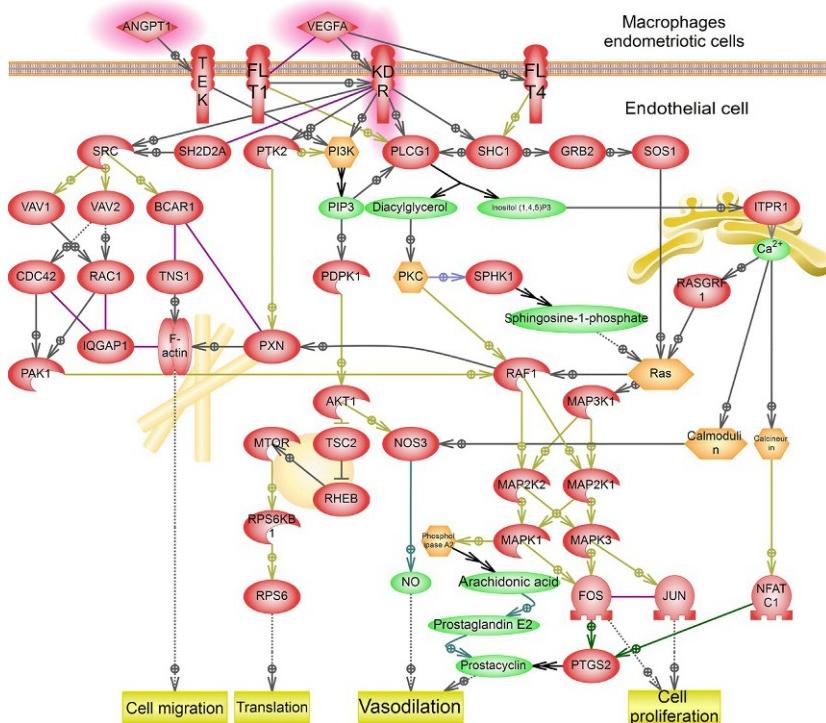


FIG. 13 Pathway 3: Enhanced angiogenesis in endometriosis.

## References

- Disease number # 131200 in Online Mendelian Inheritance in Man (OMIM database, [www.omim.org](http://www.omim.org)).
- ICD-10: disease code N80. Diseases of the genitourinary system (N00-N99). (ICD-10, <https://icdlist.com>). ICD-11: disease code GA10.
- Ahn, S.H., Singh, V., Tayade, C., 2017. Biomarkers in endometriosis: challenges and opportunities. *Fertil. Steril.* 107, 523–532. <https://doi.org/10.1016/j.fertnstert.2017.01.009>.
- Attar, E., Bulun, S.E., 2006. Aromatase and other steroidogenic genes in endometriosis: translational aspects. *Hum. Reprod. Update* 12, 49–56. <https://doi.org/10.1093/humupd/dmi034>.
- Aznaurova, Y.B., Zhumataev, M.B., Roberts, T.K., Aliper, A.M., Zhavoronkov, A.A., 2014. Molecular aspects of development and regulation of endometriosis. *Reprod. Biol. Endocrinol.* 12, 50. <https://doi.org/10.1186/1477-7827-12-50>.
- Bulletti, C., Coccia, M.E., Battistoni, S., Borini, A., 2010. Endometriosis and infertility. *J. Assist. Reprod. Genet.* 27, 441–447. <https://doi.org/10.1007/s10815-010-9436-1>.
- Bulun, S.E., Cheng, Y.-H., Pavone, M.E., Xue, Q., Attar, E., Trukhacheva, E., Tokunaga, H., Utsunomiya, H., Yin, P., Luo, X., Lin, Z., Imir, G., Thung, S., Su, E.J., Kim, J.J., 2010. Estrogen receptor-beta, estrogen receptor-alpha, and progesterone resistance in endometriosis. *Semin. Reprod. Med.* 28, 36–43. <https://doi.org/10.1055/s-0029-1242991>.
- Bulun, S.E., Monsavais, D., Pavone, M.E., Dyson, M., Xue, Q., Attar, E., Tokunaga, H., Su, E.J., 2012. Role of estrogen receptor-β in endometriosis. *Semin. Reprod. Med.* 30, 39–45. <https://doi.org/10.1055/s-0031-1299596>.
- Djokovic, D., Calhaz-Jorge, C., 2014. Angiogenesis as a therapeutic target in endometriosis. *Acta Medica Port.* 27, 489–497.
- Ferri, F.F., Ferri, F.F., 2018. Ferri's Clinical Advisor 2018. 5 Books in 1. <https://www.elsevier.ca/ca/product.jsp?isbn=9780323529570>.
- Girling, J.E., Rogers, P.A.W., 2009. Regulation of endometrial vascular remodelling: role of the vascular endothelial growth factor family and the angiopoietin-TIE signalling system. *Reproduction* 138, 883–893. <https://doi.org/10.1530/REP-09-0147>.
- Huhtinen, K., Stähle, M., Perheentupa, A., Poutanen, M., 2012. Estrogen biosynthesis and signaling in endometriosis. *Mol. Cell. Endocrinol.* 358, 146–154. <https://doi.org/10.1016/j.mce.2011.08.022>.
- Hur, S.E., Lee, J.Y., Moon, H.-S., Chung, H.W., 2006. Angiopoietin-1, angiopoietin-2 and Tie-2 expression in eutopic endometrium in advanced endometriosis. *Mol. Hum. Reprod.* 12, 421–426. <https://doi.org/10.1093/molehr/gal049>.
- Laschke, M.W., Menger, M.D., 2012. Anti-angiogenic treatment strategies for the therapy of endometriosis. *Hum. Reprod. Update* 18, 682–702. <https://doi.org/10.1093/humupd/dms026>.
- Macer, M.L., Taylor, H.S., 2012. Endometriosis and infertility. *Obstet. Gynecol. Clin. N. Am.* 39, 535–549. <https://doi.org/10.1016/j.ogc.2012.10.002>.
- McKinnon, B., Mueller, M., Montgomery, G., 2018. Progesterone resistance in endometriosis: an acquired property? *Trends Endocrinol. Metab.* <https://doi.org/10.1016/j.tem.2018.05.006>.
- Pavone, M.E., Reierstad, S., Sun, H., Milad, M., Bulun, S.E., Cheng, Y.-H., 2010. Altered retinoid uptake and action contributes to cell survival in endometriosis. *J. Clin. Endocrinol. Metab.* 95, E300–E309. <https://doi.org/10.1210/jc.2010-0459>.
- Pavone, M.E., Dyson, M., Reirstad, S., Pearson, E., Ishikawa, H., Cheng, Y.H., Bulun, S.E., 2011. Endometriosis expresses a molecular pattern consistent with decreased retinoid uptake, metabolism and action. *Hum. Reprod.* 26, 2157–2164. <https://doi.org/10.1093/humrep/der172>.

- Pierzchalski, K., Taylor, R.N., Nezhat, C., Jones, J.W., Napoli, J.L., Yang, G., Kane, M.A., Sidell, N., 2014. Retinoic acid biosynthesis is impaired in human and murine endometriosis. *Biol. Reprod.* 91, 84. <https://doi.org/10.1095/biolreprod.114.119677>.
- Rizner, T.L., 2009. Estrogen metabolism and action in endometriosis. *Mol. Cell. Endocrinol.* 307, 8–18. <https://doi.org/10.1016/j.mce.2009.03.022>.
- Rocha, A.L.L., Reis, F.M., Taylor, R.N., 2013. Angiogenesis and endometriosis. *Obstet. Gynecol. Int.* 2013, 859619. <https://doi.org/10.1155/2013/859619>.
- Sacco, K., Portelli, M., Pollacco, J., Schembri-Wismayer, P., Calleja-Agius, J., 2012. The role of prostaglandin E2 in endometriosis. *Gynecol. Endocrinol. Off. J. Int. Soc. Gynecol. Endocrinol.* 28, 134–138. <https://doi.org/10.3109/09513590.2011.588753>.
- Sokalska, A., Anderson, M., Villanueva, J., Ortega, I., Bruner-Tran, K.L., Osteen, K.G., Duleba, A.J., 2013. Effects of simvastatin on retinoic acid system in primary human endometrial stromal cells and in a chimeric model of human endometriosis. *J. Clin. Endocrinol. Metab.* 98, E463–E471. <https://doi.org/10.1210/jc.2012-3402>.
- Wieser, F., Wu, J., Shen, Z., Taylor, R.N., Sidell, N., 2012. Retinoic acid suppresses growth of lesions, inhibits peritoneal cytokine secretion, and promotes macrophage differentiation in an immunocompetent mouse model of endometriosis. *Fertil. Steril.* 97, 1430–1437. <https://doi.org/10.1016/j.fertnstert.2012.03.004>.

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## P A R T III

# Pathway analysis perspectives/advantages

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## 14

# Applications of disease pathways in biology and medicine

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Images of pathways along with their descriptions help summarize the progression of biological events and the order of complex molecular interactions in an easily understandable format. Therefore, images of the cross talk between cellular molecular cascades are useful descriptive materials for biomedical training. The most promising use of the pathway concept, however, has to do with the computational model rather than an image or visualization summary. The computational pathway model is a form of knowledge graph that contains objects (nodes) and relationships (edges or arcs) between them (more on these in Chapter 1, “Introduction”).

In addition to their names, objects, and relationships, pathway models have various annotations such as identifiers and synonyms, indications of direction, mechanism of interaction, or other attributes. Additional annotations usually rely on the database and the software application with which the pathway model was built.

For example, the adverse outcome pathways (AOPs) knowledge-base (<https://aopkb.oecd.org>, <https://aopwiki.org>) stores information on the mechanisms by which chemicals induce toxicological or other health-related adverse effects in living cells and tissues. Each AOP model describes a biological cascade of measurable events resulting from perturbation by a stressor, and it includes obligatory blocks such as the molecular initiating event (MIE), key event (KE), key event relationship (KER), and measurable adverse outcome (AO). Those AOPs that are relevant for humans are used in regulatory toxicology testing, molecular screening, and risk assessment for substances and drugs, and they are defined by standardized guidance documents (<https://www.oecd-ilibrary.org>). The concept of adverse outcome pathways is used in various applications from the classification and labeling to the identification of research priorities in general toxicity testing and assessment (Bal-Price et al., 2017).

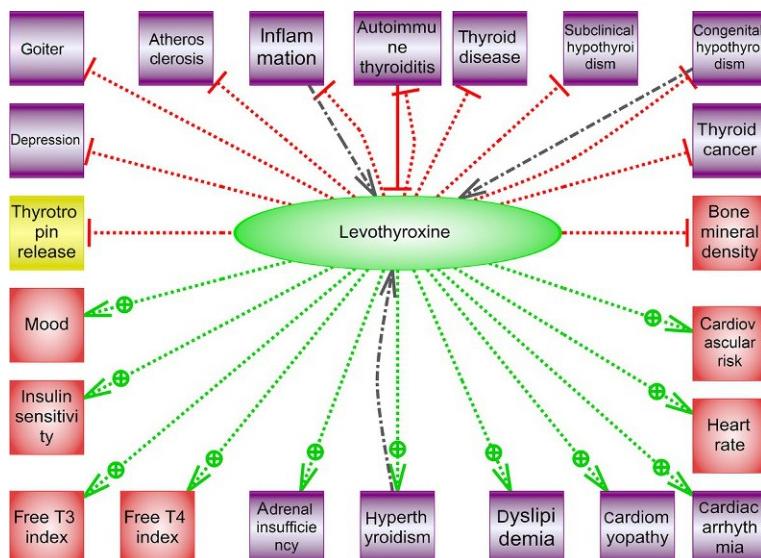
AOP is a variation of the kinetic pathway model. Kinetic models contain not only the names of objects but also their molecular concentration values. Kinetic models allow for the mathematical simulation of the dynamic behavior of molecular cascades and changes in biochemical reactions in response to various stimuli. Kinetic metabolic pathways are typically used in metabolic engineering (Copeland et al., 2012). There are other applications for kinetic pathway models, for example, to estimate the pharmacokinetics and pharmacodynamics of drugs or toxins (Lebeda et al., 2012). However, this book includes descriptions of “structural models” of human diseases rather than “dynamic models” (ElKalaawy and Wassal, 2015) (for more on dynamic models, read later).

Annotations of pathway models described in this book rely on the biomedical literature network database (more on Pathway Studio data model in Chapter 1, “Introduction” and in “Guide and Legend”). This Elsevier Pathway Studio Database (PSD or ResNet) is an example of a powerful graph database that contains more than 10 million relationships between biological concepts with supporting records from published papers (Cheadle et al., 2017). All relationships in PSD can be imaged as one giant “knowledge graph” network or map in which individual pathway models are parts of this map. Pathway models joined by such methods in one network provide numerous possibilities for using pathways in systems biology and biomedicine. For example, the disease pathways described in the book, when connected to the PSD, can be used as a bibliography for further reading and to search for published facts. Herein, while exploring the pathway, it is possible to find publications on the role of a specific gene

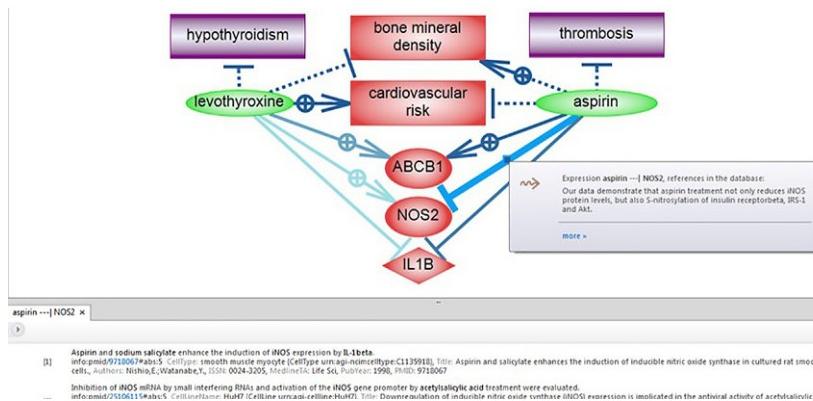
or biomarker in a disease in a few minutes. Also, with PSD, it is easy to find a list of drugs and their protein targets for a certain disease and for clinical trials or to understand adverse effects for a certain drug. Secondly, disease pathways in the PSD are useful for drug repurposing, the discovery of new applications for already approved and used drugs (see Fig. 1). Thirdly, PSD can help to review how several drugs or diseases relate to each other and which common molecular cascades they affect (Fig. 2). Therefore, pathway collections such as Elsevier Disease Pathways, as well AOPs, reduce the total time that a researcher would spend collecting and analyzing primary information and they facilitate drug research and development.

The various additional annotations within pathway models are important for the analysis of experimental data that have been acquired by high-throughput techniques such as molecular screening (microarrays) and next-generation sequencing (NGS). Naturally the analysis of patient data obtained by molecular screening techniques is the most encouraging use of pathway collections and biomedicine graph databases.

Terabytes of data from genomics, proteomics, transcriptomics, metabolomics, lipidomics, and other omics methods became available with the onset of new molecular technologies (Alyass et al., 2015; Gligorijević et al., 2016; McCue and McCoy, 2017). Microarray or exon-sequencing methods can detect a plurality of differentially expressed genes in healthy compared with pathological states. NGS technology, combined with results



**FIG. 1** Published evidences from Pathway Studio about levothyroxine effects on diseases (blue), clinical parameters (red), and cellular process (yellow). For detailed legend see Guide and Legend.



**FIG. 2** Published evidences from Pathway Studio about similar and contradictory effects of levothyroxine and aspirin. For detailed legend see Guide and Legend.

of genome-wide association studies (GWAS or WGAS—whole genome association study), may add more information regarding the patient genetic variation markers predisposing or causing a disease (He et al., 2017). Single-cell screening approaches are able to provide even more specificity to the results. High-throughput screening of chemical compound libraries against a protein target helps to discover the compound that may become the drug (Hedlund and Deng, 2018; Yu and Lin, 2016).

Bioinformatics methods can help interpret thousands of measured molecular changes, thus transferring the information into a higher-level narrative that can be easily understood and communicated by biologists. The analysis of collected patient data with the use of pathway models offers a global biological overview of molecular relationships in experimental samples. Altogether, the NGS and omics data combined with high-quality bioinformatics analysis are the basis for personalized or precision medicine in which individual and environmental sources of variability are taken into account for disease diagnosis, treatment, or prevention (Alyass et al., 2015).

### Pathway analysis for personalized medicine

Along with the advancement of high-throughput molecular biological methods, the use of the terms “personalized,” “individualized,” “precision,” or “predictive” medicine in healthcare has increased dramatically. Much overlap exists between these terms, while the term “precision medicine” tends to be the most popular today. According to the US National Library of Medicine (NIH), precision medicine is “an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person.” The

National Research Council (NRC) defines “personalized medicine” as an older term with a meaning similar to “precision medicine.” The NRC suggests using the newer term “precision medicine” to avoid possible misinterpretations of the term “personalized medicine” as a treatment developed exclusively for an individual patient, which is beyond current technological abilities ([National Research Council \(US\) Committee on A Framework for Developing a New Taxonomy of Disease, 2011](#)).

From another point of view, merging the “personalized medicine” and “preventative medicine” concepts under one term, “precision medicine” may be confusing from scientific and social points of view. Personalized medicine is focused on providing a cure to a patient, while the precision medicine initiative has to do de facto with public health and the reduction of severity and treatment costs of disease. These paradigms are different in terms of the choice of drugs, the degree of patient suffering, and clarity of the outcome. The current vision of precision medicine appears to focus on disease classification using traditional phenotypic and genetic approaches that are more relevant for disease prevention and diagnosis rather than treatment. The precision medicine approach can be useful for improving the current state of disease diagnostics and for identifying patient subgroups with similar genetic, phenotypic, and lifestyle parameters that can contribute to the disease.

A truly individualized, personalized medicine approach is still required for the broad group of patients who cannot be categorized by mainstream precision medicine or who suffer from diseases that have no effective treatment options. Oncology is the first area with examples of a truly individualized clinical approach to diagnosis and therapy ([Kotelnikova et al., 2016](#)). All modern drugs are developed for precise molecular targets and thus are assumed to be used against clearly identified molecular mechanisms of a given disease. Therefore, the disease mechanism and the affected signaling pathways in an individual patient must be diagnosed first before selecting a therapy. In this context, calling this approach either personalized or precision medicine makes no difference.

It is necessary to obtain patient DNA and/or RNA samples to start the modern precision medicine roadmap, and the collected material can be analyzed in different ways. Classic methods of cytogenetic analyses are used for the detection of large structural alterations in the genome. Screening of small mutations is not less important because even single nucleotide variants or polymorphisms (SNPs or SNVs) can lead to severe disruptions in the function of proteins and cells. From the technology perspective, previously popular methods of biochip arrays are being increasingly replaced with sequencing. Nucleotide sequencing is the nonspecific reading of the order of all nucleotides in a sample that does not require fragments of molecules with a known structure for detecting identical molecular patterns in the experimental sample.

The first thing that can be learned from the analysis of a patient's sample is the presence or absence of already known genetic variants—disease biomarkers—in her or his genome. Information on population-specific single genetic variants (SNVs) can be found in publicly available databases. There are several databases that are widely accepted to contain quality information including, but not limited to, Online Mendelian Inheritance in Man (OMIM, <http://omim.org>), ClinVar (Sequence Variation Related to Human Health, <https://www.ncbi.nlm.nih.gov/clinvar>), and the GWAS Catalog (NHGRI-EBI Catalog, <https://www.ebi.ac.uk/gwas>).

However, the statistically significant association of genetic markers with a disease depends on the population studied and the methods used in the study. It is hard to definitely answer the question of what causative variants are statistically associated with a disease or a trait, even with currently available data from hundreds of studies. The lists of genetic variants associated with the same disease but acquired in different laboratories may even be disjointed. The Human Genome Variation Society is an example of efforts being made to structure and summarize the results of thousands of studies conducted on disease associations and population distributions of human genomic variations (HGVS, <http://www.hgvs.org>). Nevertheless, with regard to clinically significant genetic markers rather than the experimental ones, there are no established standards as of today, and the number of "medically" reliable markers is low, although this area of applied science is being actively developed. Commercial services are being launched, and clinical guidelines are being published (He et al., 2017; Kotelnikova et al., 2016).

Pharmacogenomics can serve as an example of the use of known individual genetic markers to suggest the therapy. For instance, several SNVs in the thiopurine methyltransferase gene (*TRMT*) are known to cause either high or low transcriptional activity of the enzymes required for catabolizing thiopurine drugs, such as azathioprine (AZA). The allele termed *TPMT\*3A*, the most widespread in Caucasians, downregulates thiopurine methyltransferase activity by 1.6%. At the same time, *TPMT\*3C*, the most widespread variant in East Asia, causes a 17% decrease in enzyme activity. Low TPMT activity significantly increases the risk of AZA-related adverse effects including leukopenia, myelosuppression, and bleeding. That is why screening for the level of TPMT activity is recommended before initiating therapy with AZA.

Polymorphisms that impact absorption, distribution, clearance, or the kinetics of drugs, such as the ones in the *TRMT* gene, are called ADME gene variants (for absorption, distribution, metabolism, and excretion). Polymorphisms in the cytochrome P450 enzymes with regard to maintaining effective therapeutic doses of warfarin and tamoxifen are another well-known example of ADME gene variants. However, aside from those

illustrative examples, the number of known ADME biomarkers barely exceeds 100 ([Tremaine et al., 2015](#)).

Most of the research that can be attributed to personalized medicine focuses on mutation analysis and diagnostics. However, the analysis of individual expression profiles can also be used in diagnostics and in the selection of treatment. For example, the successful struggle of oncologist Lucas Wartman with his own diagnosed acute lymphoblastic leukemia ([Wartman, 2018](#)) was based on the ability to identify that the *FLT3* gene was overexpressed in his leukemic cells. This knowledge determined the choice of drug that, despite the fact that it is usually administered to treat other types of cancer, turned out to be effective. We must say though that such cases are as inspirational as they are rare. Complex changes that occur due to interactions between genes and proteins are difficult to identify, and it is also hard to determine the precise cellular mechanism being affected by the drug and which target mechanism can be influenced to alter the course of the disease. Dr. Wartman himself states that he was incredibly fortunate that the selected drug was effective ([Wartman, 2018](#)).

Pathway analysis is aimed at the assessment of molecular systems rather than the individual genes typically assessed in high-throughput screening tests. This approach is promising since the disease may originate from different combinations of multiple mutations in functionally related groups of genes and changes in the activity of interacting proteins. The gene candidate method is not effective in the case of diseases with genetic heterogeneity, for example, the oncological diseases. A flat list of genes with mutations or with altered expression does not have much value for diagnostics and for the selection of therapy without taking the molecular interactions into account. Pathway analysis can help connect affected molecular blocks in one system and thus allow scientist and clinicians to predict how cellular functions are altered.

Pathway analysis of NGS or microarray expression results can help find new applications for known drugs by discovering nontypical disease signaling pathways activated in any given patient. Pathway analysis may also contribute to the development of personalized drug therapy by estimating the benefits of drug combinations. Obviously, networks and pathway models that depict physical interactions between compounds and their target proteins are especially useful for the selection of drugs with minimum unexpected side effects and for new drug development in general.

It must be noted that the future development of methods in personalized medicine assumes a combination of several data types (for example, genetic variations and alterations in gene expression) and several bioinformatics approaches to analyze and concretize the patient's status.

## Bioinformatics foundation for patient data analysis

Ultimately the approach to a bioinformatics analysis of patient data depends on the nature of the data and on the choice of biological question to be asked and the associated hypothesis to be tested. In general the analysis implies a variety of statistical or algorithmic methods for discovering patterns in the measured activities of molecules and associating them with molecular functions, biological processes, or diseases. The revealed results are expected to explain how conditions (for example, disease) alter the molecular characteristics of the sample.

During the first step of the analysis, the results of expression arrays and DNA sequences must be converted into a list of genes with known names and identifiers. The annotation of gene names and identifiers is a challenge in the analysis of high-throughput expression assays and sequences. Proper mapping of sequences, names, and identifiers from experimental data with ones used in publicly available biological databases (primary in the NCBI Gene (Entrez) database) is broadly used to obtain a preliminary picture of the functional roles of genes ([Henderson-Maclennan et al., 2010](#); [Hung et al., 2012](#)).

The normalization and preprocessing of measured raw numbers of gene expression levels is the most challenging in the analysis of microarrays. Normalization removes noise from measurement errors or heterogeneity in compatible samples. Normalization is often the most critical and problematic step in bioinformatics analysis because it is difficult to separate the true meaningful biological variability from experimental noise or from irrelevant deviations in the distribution of numbers. Identifying the type of distribution is the problem itself, as biological heterogeneity comes from the complex dynamic nature of molecular interactions with feedback loops and temporal associations. With the aim of increasing confidence in the chosen normalization method, multiple measurements of the same gene are desired. Also, the presence of multiple samples with controls, such as samples from healthy individuals, is essential ([Alyass et al., 2015](#)).

The overwhelming majority of approaches use differentially expressed genes as the input for the analysis of microarrays. In this case the analysis of expression arrays (after normalizing for molecular annotation and numbers) starts with calculating the statistical difference between measured numbers indicating the proportion of the same molecule in two samples, for example, in health and disease. The differential expression analysis uses standard statistical methods such as parametric and nonparametric tests to replace the raw (intensity-based) units of gene expression with probability values (*P*-values) of fold change comparative ratios ([Smyth, 2004](#)). Classic statistics are sensitive enough to detect differences in the expression at the population level. To precisely detect contrasting gene activity at the individual, patient-specific level,

a sufficient number of gene expression values from “healthy” samples have to be previously collected (Wang et al., 2015). Another strategy involves integrating multiple omics experiments or even different types of experiments into one single dataset. The interpretation of differential expression values and any other statistics-related numbers should be done carefully as many of them are usually based on assumptions that are not relevant to the biology of the particular experiment. For example, merging of transcriptomic and proteomic profiles is not valid in eukaryotes (Alyass et al., 2015).

Later, we discuss techniques that were initially designed for the analysis of expression arrays to evaluate the biological roles and functions of measured molecules. However, the methods might be extended to nucleotide sequence data (Kao et al., 2017). In the context of the analysis of the functional roles of genes, there is a problem with single nucleotide variants (SNVs) in that not all of them can be an attribute of (or map to) a distinct gene because SNVs may occur in noncoding regions. Also, there can be novel SNVs or SNVs for genes with unknown functions. Many authors assign unmapped SNPs to all genes within a distance window, ranging from 10 to 500 kb. Researchers interpreting the results should be aware that some SNPs may not be functionally related to their assigned gene(s) (Ramanan et al., 2012). During the analysis of single variants and sequence array data, one should also consider another problem of gene annotation. Whereas expression arrays return a single signal for each gene, genetic variation arrays may include multiple signals per gene because one detected DNA variant can be assigned to several genes. However, typical statistical analyses can utilize only one probabilistic *P*-value association per expressed gene or per one genetic variation associated with a phenotype (Peterson et al., 2013; Aslibekyan et al., 2014).

The purpose of this chapter is to provide the reader with an introduction to the bioinformatics analysis of patient data and to provide a warning about the potential risks associated with these analyses. The main risk is the overestimation of the power of statistics and getting false-positive results and invalid findings with regard to their correlations with biological functions.

## Gene grouping techniques

It is difficult to interpret the biological meaning of the results of analyzing microarray or sequencing data from a flat list of thousands of differentially expressed genes or of discovered genetic variants even when they are ranked by *P*-values. Further computational analysis is needed to switch from the list of genes to their functional effects and to the cellular processes or pathologies that are regulated and affected by measured genes. The “gene-by-gene” interpretation will not entirely uncover these

processes because each cellular event is controlled by multiple genes interacting with each other. Splitting one single list of genes into meaningful groups is the primary purpose of omics data analysis, which may reveal a biological sense of the experimental results ([Tarca et al., 2006](#)).

Classification and clustering methods as part of descriptive statistics are actively used for grouping genes with similar expression patterns. The supervised classification (machine learning and class prediction) technique places genes with similar expression levels or patterns into preexisting categories, while unsupervised classification (class discovery and clustering) calculates a number of homogeneous categories into which genes can be placed ([Allison et al., 2006](#)). There are different clustering algorithms although they all are based on straightforward statistics and, in general, on calculating the distance (dissimilarity) that is used for recognizing the relationships among gene groups and the similarities that are used for the qualitative comparison of gene clusters. K-means-type clustering, hierarchical clustering, and model-based methods of self-organizing maps (SOM) are the most often cited clustering algorithms in microarray analysis. More on clustering methods can be found in ([Oyelade et al., 2016](#); [Xu and Tian, 2015](#)).

Classification techniques can be applied to experimental results without any prior knowledge on the functions of interesting genes. For example, the approach of clustering individual gene expression levels measured over time (or with other conditions) is applied for the *in silico* modeling or the reconstruction of putative dynamic networks of gene-to-gene interactions directly from the data without any external knowledge. Boolean, Bayesian, and neural networks; differential equation models; and other methods are used for the construction of networks from gene expression data alone ([Ay and Arnosti, 2011](#)).

However, since classification provides results based only on statistical characteristics of expression data, the clustering results still require the interpretation of hidden biological processes, and often, they remain meaningless. Practice has shown that prior knowledge in the form of external and previously created lists of protein families, gene functional groups, and pathways allows the validation and discovery of more biologically meaningful clusters ([Okada et al., 2005](#); [Peng et al., 2014](#)).

Any ontology with genes or proteins annotated with distinct biological roles and the collection of pathways can be used as the external biological knowledge applied to the analysis of microarray or sequence data. The Gene Ontology (GO) is the most often used free source of structured knowledge on functional molecular activities. GO includes protein and gene names grouped under terms of different molecular processes and functions that are themselves organized into a hierarchical structure ([Ashburner et al., 2000](#); [Gene Ontology Consortium, 2017](#)). GO consists of three main vocabularies: molecular function (MF), biological process (BP),

and cellular component (CC). GO refers to information about the roles of each annotated gene product. Each GO term has hierarchical relationships with its “parent” and “child” neighbors so GO can be represented in the form of a network.

There are several examples of combining clustering techniques with preselected GO gene groups to identify coexpressed genes in experimental data by overlapping their functional roles and getting more reliable biological results. However, most of the studies in this area have been done on data from animals and yeast (Acharya et al., 2018; Paul and Shill, 2018).

Gene Ontology expansion starts in the “gene-class testing” direction of the analysis of microarray data (Allison et al., 2006), which is now the mainstream and is broadly used technique for grouping genes from microarrays data. “Gene-class testing” methods involve probability-based statistical approaches from estimation theory that, in general, tests a null hypothesis that the chosen group of genes is overrepresented in the list of differentially expressed genes (Goeman and Bühlmann, 2007). Tested groups of genes are usually named gene sets and are defined a priori, coming from external resources like GO. Pathways derived from these methods and from clustering analyses were originally used as simple gene sets—a list of genes without information about their interactions or other pathway annotations. Later, algorithms that use all available information from the pathways were developed.

Although the fundamental methods of statistical hypothesis testing are similar, today, the number of publications with different variations and different techniques is close to several thousands. This is also true with other types of biomedical data analysis. So many tools have been developed that there are commercial services that offer to identify bioinformatics trends and provide a summary of who has done what (see, e.g., omicX, <https://omictools.com>).

Noticeably, there is no mature classification and widely accepted terminology for the types of high-throughput statistical analysis as the area is still evolving (Alyass et al., 2015; García-Campos et al., 2015; Khatri et al., 2012; Nam and Kim, 2008; Ramanan et al., 2012; Wu and Lin, 2009).

As of today, many authors recognize three principal types of “gene-class testing” analysis or “functional enrichment analysis,” which are overrepresentation or enrichment analysis (ORA), functional class scoring (FCS), and pathway topology (PT) as suggested by Khatri, Sirota, and Butte (García-Campos et al., 2015; Khatri et al., 2012; Manoli et al., 2006; Mitrea et al., 2013). In addition, the classification of methods in bioinformatics analyses can be a difficult task due to the fact that researchers use various combinations of statistical methods and logic workflows (Ackermann and Strimmer, 2009). These combinations may vary to different degrees, at least by the peculiar names that authors gave them, as BubbleGUM (Spinelli et al., 2015) or LEGO (Dong et al., 2016). Several reviews are

devoted to evaluating and comparing the power of existing variations of functional enrichment analyses (Hung et al., 2012; Yu et al., 2017).

Sometimes the methods listed in this chapter and similar ones aimed to find biological functions and processes associated with the experimental results are called “pathway analysis” or “network analysis,” and these terms are used synonymously. We tend to use the term “pathway analysis” to define techniques that use *a priori* generated pathways of any format. We do not use the term “pathway analysis” for methods that infer constructed de novo models of interactions from experimental measurements (cluster analysis) or that rely on the database where all members are connected in one big network (network analysis).

To summarize the analysis of molecular screening results is still a challenging task despite plenty of available bioinformatics techniques. There are two different types of techniques commonly used: reconstructing pathways or coexpression networks from the data and detecting patterns in the data that align with previously collected knowledge about gene function. These techniques can help generate nice charts, but those charts will be of little value without the ability of the researcher to formulate the right question in the first place.

### Pathway analysis as a part of functional enrichment analysis

Functional enrichment analysis is driven entirely by candidate sets (groups) of functionally related genes, that is, by the application of prior biological knowledge and preselected hypotheses about gene function. The gene sets here are collections of pathways or names of molecules categorized in groups by specific biological functions. The researcher can create gene sets and pathways required for the analysis, but usually, they are retrieved from publicly available resources including the most used resources such as GO; Kyoto Encyclopedia of Genes and Genomes (KEGG); or Molecular Signature Database, MSigDB (read more on these later).

Pathways can be considered typical gene sets where only the pathway’s name is used and a chain of interactions within the pathway does not influence the analysis. According to the classification of methods of functional analysis that subdivides them into overrepresentation (ORA), functional class scoring (FCS), and pathway topology (PT), only PT uses the pathway as a model of molecular interactions. Many variations of ORA and FCS use pathways exclusively as a list of molecules, gene sets, which those methods try to align with experimental data. Such methods, strictly speaking, should be named “gene-set analysis” (GSA) or in a broad sense “functional enrichment analysis” rather than “pathway analysis.”

Although the algorithms used in functional enrichment analysis are diverse, they share common features and are guided by the same probability-based statistics.

Firstly, *P*-values can be calculated at three levels: the single gene level, the gene-set (pathway) level, and the sample level (Ackermann and Strimmer, 2009; Hung et al., 2012; Lin et al., 2018). While gene-level statistics are usually calculated based on fold change of expression levels, *t*-statistics, correlation coefficient, ANOVA, or linear/logistic regression, gene-set statistics include the median of single gene expression level calculations, Kolmogorov-Smirnov test, the maxmean statistic, and the Wilcoxon rank sum test.

Then, as several researchers point out, there are two or three basic null hypotheses that are being used to test functional enrichment analysis. An understanding of how to formulate the null hypothesis properly is very important for conducting the statistical analysis of biology-related numbers since it is closely related to the scope of the experiment. Based on the type of null hypothesis, functional enrichment analysis can be roughly divided into methods that use the first “competitive null hypothesis” (Q1), the second “self-contained null hypothesis” (Q2), or the “nested null hypothesis” (Q3) (Alexeyenko et al., 2012; García-Campos et al., 2015; Goeman and Bühlmann, 2007; Maciejewski, 2014; Nam and Kim, 2008). Q1 or competitive null hypothesis tests inquire whether the genes in the pathway or the gene set have differed from all other genes in the experiment. Methods that use Q1 are currently the most prevalent.

Q2 or the self-contained null hypothesis method compares only genes in the fixed pathway or the gene set, regardless of all genes in the experiment. Q2 assumes that there is no association between genes in a pathway. Q1 and Q2 validate the significance of individual gene sets. The “nested null hypothesis” (Q3) method compares genes in the pathway or the gene set with the entire dataset (all genes both inside and outside of the gene set). Authors who have suggested this classification, for example, Goeman and Bühlmann, strongly recommend using self-contained methods (Goeman and Bühlmann, 2007). In contrast, other authors see an advantage in the competitive approach (Kao et al., 2017; Maciejewski, 2014). It is worth adding that the choice of the null hypothesis for testing and the method used for the analysis should depend on the experiment’s design and biological sense, which often lead to the need to test a combination of the hypotheses (Emmert-Streib and Glazko, 2011; Tian et al., 2005).

Historically the overrepresentation or enrichment analysis (ORA) was the earliest method used for the analysis of microarrays. ORA methods evaluate the significance of overlap between the chosen fractions of experimental results with genes from each gene set within a pathway. Methods from the ORA class take a part of the list of differentially expressed genes as input and generate as output a list of groups of genes with different functions from a chosen external catalog of such groups. The researcher decides what part of the group of differently expressed genes will be analyzed by setting the cutoff threshold for the calculated *P*-value. For

example, only genes with more than twice the difference in expression levels can be chosen. ORA uses simple statistical tests (Fisher's exact test, hypergeometric distribution, binomial distribution, chi-square distribution, etc.). The most relevant pathways will be marked with the best (lowest)  $P$ -value in the final list of all analyzed gene groups.

ORA methods have many limitations such as the biased cutoff for choosing preselected parts of the differentially expressed genes and the assumption that gene sets and pathways are independent of each other, which is not true in real life. Also, ORA grouping methods ignore genes that are not included in the preselected part of the list of differentially expressed genes, thus increasing the chances of missing a biological signal. Moreover, it is believed that in many diseases changes in the levels of gene expression are moderate and undetectable for individual genes ([Emmert-Streib and Glazko, 2011](#)). ORA methods are focused mainly on finding associations with GO gene sets and less with pathway collection, as it can be done with one of the popular tools, GoMiner ([Zeeberg et al., 2003](#)). There are many reviews of ORA methods ([García-Campos et al., 2015](#); [Khatri and Drăghici, 2005](#); [Rivals et al., 2007](#); [Sun et al., 2018](#)).

ORA can also be named individual gene analysis ([Nam and Kim, 2008](#)) because it evaluates the overlap of individual genes between two lists: the differentially expressed genes and genes that share common biological themes. Methods of the next category, FCS, evaluate microarray data at the level of the whole pathway and gene set.

Functional class scoring methods comprise a broad category that is united by the idea that even weak alterations in gene expression are important if they are coordinated between sets of functionally related genes and pathways. To implement this idea, FCS, in a manner similar to ORS, works with external gene sets but uses the whole list of measurements from an experiment and the values of all differentially expressed genes without arbitrary  $P$ -value cutoff limitations. FCS methods have several steps. Usually, first gene-level statistics are calculated, then they are aggregated into a single pathway-level statistic, and then the significance of the pathway-level statistic is measured. The last step strongly depends on the tested type of null hypothesis (competitive or self-contained). FCS methods are useful in identifying disease specific genes which experimental expression does not change at a statistically significant level. FCS methods also allow the detection of more biological topics relevant to the experiment, although questions remain regarding the detection of false-positive patterns. ([Khatri et al., 2012](#)).

Most of FCS methods do overcome  $P$ -value cutoff limitations, but similar to ORA, they analyze each pathway independently without considering interactions among pathways ([Pavlidis et al., 2004](#); [Wu and Lin, 2009](#)).

Gene-set enrichment analysis, GSEA ([Subramanian et al., 2005](#)), is the most cited method in this group. Actually the term FCS is not very

popular and is being replaced by some authors with “GSEA algorithm and related methods” (Ackermann and Strimmer, 2009). GSEA was developed by Mootha and coauthors (Mootha et al., 2003), and it is maintained by the Broad Institute (<http://www.broadinstitute.org/gsea>). The goal of GSEA is to determine whether genes in any gene set from an a priori defined collection are overrepresented at the extremes (top or bottom) of the list of differentially expressed genes. In the first step, GSEA ranks all experimental genes in the dataset based on the fold change of their differential expression. In the second step, GSEA calculates an enrichment score that reflects the degree to which a gene set is overrepresented at the top or bottom of the list of experimental genes. The enrichment score corresponds to a weighted Kolmogorov-Smirnov-like statistic; it is defined as the maximum distance from the middle of the ranked list. If genes from a gene set or pathway are not randomly distributed across the datasets, they are expected to be coregulated genes that tend to share similar expression patterns that have correlations with this particular gene set and the phenotype that it describes. Competitive or self-contained null hypothesis testing is used in GSEA to check the significance of possible correlations.

As usual, in the process of new bioinformatics method development, a unique GSEA software package creates a separate collection of gene sets (Molecular Signature Database, MSigDB) for the analysis. However, other collections and software environments can also be used.

Currently, there are many combinations, extensions, and improvements to the classical GSEA algorithm. Also, there are many publications that attempt to evaluate the power and accuracy of GSEA, GSEA-related methods, and their comparisons with other approaches (Glazko and Emmert-Streib, 2009; Hung et al., 2012; Liu et al., 2007a; Maciejewski, 2014; Manoli et al., 2006; Mathur et al., 2018; Ramanan et al., 2012).

### Pathway topology–based enrichment analysis

All the methods discussed earlier use pathways merely as a group of genes because they do not consider interactions between the genes on the pathways; rather, they only use the names of genes and the names of pathways to identify a significantly affected biological point. In contrast, pathway topology (PT)–based methods repeat the FCS methodology except they use the structure of the pathway model, including order, directions, and types of molecular interactions, to compute gene-level statistics. Several algorithms that consider elements of the mathematical graph theory where the pathway is considered as a graph with nodes and edges have been developed implementing this idea. Also, probabilistic statistics are actively used by PT methods to compute node-level (similar to gene-level) ranking. The “perturbation factor” is used as a score to evaluate the significance of the observed possible rearrangements of the

pathway members (Tarca et al., 2009). In general, PT methods for node-level ranking use (1) the measure of centrality (closeness) of the node, that is, connectivity of molecule on the pathway, for example, a gene to all other nodes of the model; (2) the similarity of clusters of differentially expressed genes within the pathway; and (3) probabilistic values of the random distribution of nodes by comparing the effects of interactions (edges) between them (Mitrea et al., 2013).

The direction of molecular interactions and the effects of the interactions (positive or negative) are critical topological features of the pathway that hold the information regarding emitters and receivers of the signal from genes with differential expression levels. Also, this knowledge allows linking up- or downregulated differentially expressed genes with the activation or inhibition of the pathway (Haynes et al., 2012).

The next step is the computation of a significance “score” for each pathway that reflects the association of the pathway with experimental data. To aggregate gene-level scores to pathway-level statistics, most of the PT methods use summation scoring, weighted gene-set techniques from FCS methods, or other methods specific to network-topology analysis (such as Bayesian networks, or ranking interactions (edges) instead of nodes). PT methods use approaches similar to FCS to evaluate the significance of calculated pathway scores although some of PT methods do not use this last step of functional enrichment analysis at all (Mitrea et al., 2013; Nguyen et al., 2018).

One of the earliest methods involving the pathway topology approach was signaling pathway impact analysis (SPIA), which combines standard overrepresentation tests with a measure of relative gene locations (perturbation) on a given pathway (Tarca et al., 2009). The combination of multiple techniques makes it difficult to generalize the PT class of methods similar to the FCS class. This task is further complicated by the strong dependency of PT methods on distinct characteristics of pathway topology and annotations that are themselves specific to collections of molecules. Many software applications implementing PT methods depend on the specific data structures and file formats that store pathway data and are integrated into the algorithms. Only a few tools can work with several formats and therefore work with pathways from several resources. For example, “EnrichNet” works with most popular publicly available pathway collections, and it also allows the inclusion of custom-built pathways in the analysis (Glaab et al., 2012). A recent overview of the PT class of methods with useful guidelines for method selection was published by Ihnatova and coauthors (Ihnatova et al., 2018).

Pathway topology methods are the most comprehensive methods in pathway analysis, but they have still not overcome the limitations of ORA and FCS methods regarding pathway independence. Also, PT still cannot help detecting the cross talk between pathways when the downstream

ends of one pathway can provide input signals for another one. Other common limitations of pathway topology methods go beyond the limitations of statistics, and they represent new challenges for both pathway collection construction and analysis. For example, the inability of existing methods to consider the types of cells for which the pathway was built (García-Campos et al., 2015; Khatri et al., 2012). Further, the majority of enrichment methods do not work with isoforms, splicing variants, or even single genetic polymorphisms since pathways and gene-term ontologies are usually based on the unified gene (or protein) concept. Diving into these challenges will open new possibilities and help predict changes in biological functions and help develop a list of biomarkers related to certain molecular and environmental changes.

## Network-based enrichment analysis

Functional enrichment analysis depends on *a priori* defined gene sets and manually built pathway collections for the interpretation of omics data. On the one hand the curation of data by experts ensures the quality and meaningfulness of any discovered biological processes and functions that are affected in the experimental data. On the other hand, there is no pathway collection of human data that are so comprehensive that it contains all possible functions on the molecular level with different anatomical specifications. The dependence on previously constructed pathways does not allow it to go beyond the limits of known functional roles and may be considered a disadvantage.

There have been many attempts to solve this problem, and the analysis of molecular interaction networks rather than directed signaling pathways is one of the most promising tactics. Several methods try to use automatic algorithms to reconstruct such networks directly using the results of high-throughput experiments (coexpression networks) or to aggregate them from curated databases about physical or functional molecular interactions (PPI, protein interaction networks). Networks are less likely to be biased than manually constructed gene sets and pathways (Vella et al., 2017). Although pathways combined with each other can also be used as a “networks of networks” database.

Networks can be created in different ways. “Coexpression networks” are constructed directly from experimental gene expression data or from publicly available depositories of genome-wide gene expression experiments (e.g., Gene Expression Omnibus and ArrayExpress). This approach assumes that genes with “similar” expression levels or expression patterns are related to each other. Coexpression networks constructed in this way are used to quantify pathway correlations (Barbosa et al., 2018; Pita-Juárez et al., 2018; Shojaie and Michailidis, 2010; Vella et al., 2017). Protein-protein interaction (PPI) networks are built based on either physical interactions

extracted from specialized databases or on functional, regulatory interactions that can be predicted or extracted from published articles (more about network types in [Chapter 1](#), “Introduction”). Transcriptional, metabolic, and direct physical protein-protein interaction studies provide the information for PPI network construction ([Kim et al., 2010](#); [Liu et al., 2015](#)) (also see <https://www.encodeproject.org>). Regulatory (functional) links that connect molecules with each other and with biological concepts can be extracted from the literature, for example, by NLP technology ([Egorov et al., 2004](#)). Another example is when a network is combined from other databases rather than created from experimental results or from the literature ([Himmelstein et al., 2017](#)). Apparently, it is beneficial to integrate genomic, transcriptomic, proteomic, and metabolomic measurements and prior biological knowledge from the scientific literature with pathways reconstructed manually into the network building process.

Methods that use networks to find gene groups enriched by experimentally studied genes are considered to belong to the PT approach ([Alaimo et al., 2017](#); [Mitrea et al., 2013](#)). However, we believe that those methods are suitable to be placed in the individual class, “network enrichment analysis” ([Henderson-Maclennan et al., 2010](#)). The reason for that is that these methods are capable of estimating pathway cross talk and of dealing with a new type of external data source—network databases. Network-based analysis overcomes the limitations of functional enrichment analysis by using information about interactions between pathways, instead of treating them as independent entities.

The rationale underlying network analysis is that genes that are located within a short distance in the same subnetwork (subpathways, clusters, gene modules, etc.) are likely to be involved in similar biological processes. The idea here is the same as for PT methods, to test overrepresentation of experimental genes in a subnetwork based not only on gene overlap statistics but also on gene interactions. From the very beginning, methods of network enrichment analysis like NEA ([Alexeyenko et al., 2012](#)), GNEA ([Liu et al., 2007b](#)), and SNEA ([Sivachenko and Yuryev, 2007](#)) integrate gene expression data with noncurated automatically created molecular networks and with curated gene sets. Most of the methods use previously created external networks as an information source in addition to pathways for estimating the validity of distributing experimental data by functional groups.

All network-based methods focus their attention on attempts to incorporate information from the “global” network, but each uses different approaches to quantify enrichment scores of pathways ([Braun and Shah, 2014](#)). For calculating the enrichment score, NetGen, NetPEA, CrossTalkZ, BinoX, and other methods that work with networks use techniques similar to PT class methods, such as counting the number of links between members of subnetworks, “closeness,” similarity, and others ([Braun and](#)

Shah, 2014; Ogris et al., 2017; Pita-Juárez et al., 2018; Signorelli et al., 2016; Sun et al., 2017). Network-based methods can detect subpathways that are strongly associated with experimental genes, even when they share only a few genes (McCormack et al., 2013).

Previously assembled networks can be also used to identify the functional roles of genes from the simple list of gene names regardless of differential expression values. To achieve this, tools like Pathway Studio have different options for connecting genes from the gene list to the path based on the incorporated global network of gene interactions. NetVenn is an example of a free tool that specializes on this approach that allows the easy comparison of lists of genes and places them on the interactome network (Wang et al., 2014).

Next, some of the network-based methods can detect “cross talk” between pathways, by focusing on the process of selecting significant submodules within pathways. PT methods score subpathways that are separated from the whole pathway to estimate significant overlap and to identify connections between gene clusters within the pathway (Alaimo et al., 2017; Donato et al., 2013; Ozerov et al., 2016). Network cross talk analysis connects pathways and subpathways in one network that may provide new information about the hierarchy of functional changes regulated by genes with altered expression. PathNet, PCxN, SPECifIC, CrossTalkZ, and SNEA are examples of methods that consider all possible edges in the global network to determine relationships between pathways (Alaimo et al., 2017; Dutta et al., 2012; McCormack et al., 2013; Pita-Juárez et al., 2018; Pyatnitskiy et al., 2014). PathNet suggests using directed interactions between differentially expressed genes rather than using the overlap of genes to describe the associations between pathways (Dutta et al., 2012). However, the direction of molecular interactions within networks is not acknowledged by all network-based methods (Liu et al., 2017).

The inverse task of combining pathways into a network, namely, splitting big networks into clusters (modules, hubs, or subnetworks), has been intensively exploited. The identification of subnetworks was used to model and create hypothetical pathways, to predict possible new interactions, and for phenotype classification. For example, Zhou and coauthors used molecular networks to evaluate clinical phenotypes and molecular profiles to create disease taxonomies (Zhou et al., 2018). The application of networks and pathways for disease and drug research was reviewed recently (Hao et al., 2018).

Many methods described earlier can be extended to the analysis of sequencing data (Aslibekyan et al., 2014; Holmans, 2010; Mooney et al., 2014). Also, there are many methods developed explicitly for NGS technology that are aimed at applying pathway and network analyses to identify functional gene groups and pathways that are enriched with mutated genes (He et al., 2017; Ramanan et al., 2012; Soneson and

[Delorenzi, 2013; Wang et al., 2017](#)). Pathway collections, networks, and enrichment analyses may be the answer to the challenge of the distinction between driver and passenger mutations. Passenger mutations comprise the majority of somatic genetic variations that can be detected by NGS technology, but they are not the main triggers of diseases. Estimating the degree of a genetic variant's impact on the pathways and ranking the former according to the likelihood of its effect on functional changes move NGS analysis outside of weak associations between polymorphisms and gene functions and into the role of exposing exact mechanisms ([Zimmermann, 2018](#)).

One of the main goals of patient data analysis in personalized medicine is to connect a patient's molecular profile measured in laboratory with specific cellular and system processes that are responsible for the manifestations of a disease. Only a multilevel combinatorial approach can reach this goal successfully ([Seifert et al., 2005; Werner, 2008](#)). The ideal pathway analysis method would integrate evidence from the patient's DNA sequence, gene expression data, and epigenetics with accurate and predefined pathway collection and with extensive network information about all possible molecular interactions.

### Pathway and network analysis output interpretation

The first result of omics data analyses is a list of genes with mutations or with information regarding changes in the expression of genes in the experiment or in the disease. In the next step the detection of the underlying pathways and subnetworks addresses the problem of deriving a biological interpretation of those gene lists.

However, the final result of a multistep functional enrichment analysis is just a list of names of biological processes (from 1 to 100) like "cell migration" or "insulin secretion regulation by glucose." Those are the names of manually constructed pathways and curated groups of genes. There could also be names of diseases or mutations or drugs or other terms from ontologies that are linked to genes or proteins. The result of methods that utilize additional knowledge in the form of interactions between objects in the pathway is the list of subnetworks (subclusters) with the names of connected molecules.

All of this information represents the biology of the experimental data and should be further interpreted based on knowledge of different areas of expertise including medicine or pharmacology. The literature offers multiple examples on how the bioinformatics analysis of collected samples from patients was used to identify affected mechanisms in a particular disease ([Kavanagh et al., 2013; Mejía-Pedroza et al., 2018; Muñoz García et al., 2018; Wang et al., 2019](#)).

In output lists, the top gene sets, pathways, and subnetworks are usually ordered by confidence values such as *P*-value or other scores. Except for the list the output result may be visualized in different formats from pathway maps to ontology trees, to heat maps, or to any other charts and infographics.

The problem of interpreting expression array experiments using individual signaling pathways lies in the nature of cellular molecular control. Firstly the majority of human disorders or cellular processes are rarely caused by the activity of a single gene or even a single signaling molecular cascade, but rather, they have complex combinatorial genetic, epigenetic, and metabolic causes. Gene expression is usually controlled by several sequential layers of regulators. This complexity is hard to describe with one single pathway. Even if the enrichment analysis comes up with several adjacent pathways, biologists will need to interpret them on their own.

The idea of two classes of players—"modifiers" and "specifiers"—is actively used to unravel the tangle of mutual molecular relationships (Houlston and Tomlinson, 1998; Lehner, 2007). Specifiers are causative factors, genes with mutations, elements that play a key role in the signaling or determinants that define the disease. A modifier is a controller or supervisor of molecules that causes the process. The concept of a modifier is the same as the concept of a "hub" or "seeds" that is used in network-based analysis when searching for general regulators of experimental gene expression. Also the concepts of "modifier" and "specifier" intercross with concepts of "driver" and "passenger" mutations that do or do not promote the development of complex human diseases like cancer (Haber and Settleman, 2007; Stratton et al., 2009). Because several causative genes in most cases provoke a particular cellular process or disease, there is a high probability that there is at least one common modifier for them. Common regulators and coordinately related gene clusters, in case they are identified, allow the recognition of primary biomarkers and pharmacological targets. Moreover, the concept of regulators and their targets in the network is used to investigate the genetic complexity of a particular disease and for the discovery of disease-disease associations (Kontou et al., 2016; Lehner, 2007).

Surely the precise type of interaction network is important for the accurate prediction of regulators of protein function or changes in gene expression. RNA level measurements are best paired with a transcriptional regulatory network that consists of molecular interactions between a transcription factor and its target genes. Metabolic and protein-protein physical interaction networks and pathways have information that probably is not observable on the transcriptome that itself detects changes only in mRNA levels (Mieczkowski et al., 2012). Gene expression regulatory relationships can be derived from experiments, genomic regulatory sequences (enhancers and promoters), databases of transcription factor binding sites (TFBS), and literature mining (Werner, 2008).

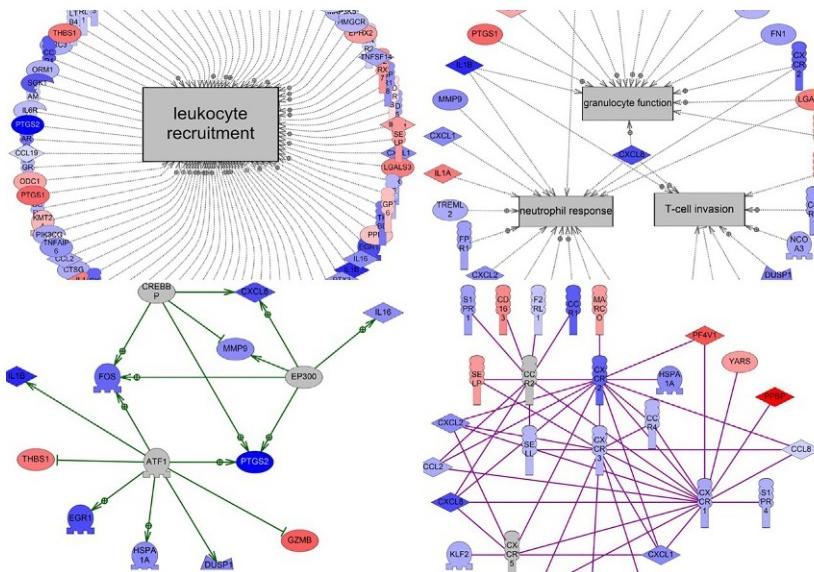
For example, the results of SNEA can include several disease pathways like the ones described in this book with marked molecules that are critical regulators of gene expression from patient sample data. The regulators can be not only transcription factors but also drugs that are known to stimulate or inhibit the activity of statistically significant cascades. Selected drugs can subsequently be prescribed to a patient.

SNEA, as implemented in Pathway Studio, combines elements of functional enrichment analysis (GSEA), which is based on GO gene sets, and prereconstructed pathways with network analysis based on the ResNet database ([Pyatnitskiy et al., 2014](#)). In the first step, SNEA creates a set of “subnetworks” from the database. Each subnetwork consists of a single “regulator” or “seed” and its nearest neighbors (“targets”) in the given direction of interactions. A user can define the direction and the preferred type of interactions and a biological type of “regulator” for the de novo calculation of the subnetwork ([Sivachenko and Yuryev, 2007](#)).

Next, SNEA uses the Mann-Whitney ranking test to evaluate the overlap of measured gene expression values in the subnetwork against the entire microarray; this is similar to the GSEA approach. Therefore, the output of the SNEA algorithm shows generated subnetworks sorted by *P*-value, each consisting of one common regulator that controls the group of a patient’s genes with similar differential expression patterns. SNEA can calculate different categories of “major regulator” and “major target” subnetworks based on the type of control and nature of the regulator.

A regulator for a subnetwork can be a gene, a protein, a protein family, a compound, or even a process or a disease. The type of control depends on the chosen types of relationships present in the ResNet network, for example, the physical interaction type—“promoter binding” control. The “major target” subnetwork category includes common effects, processes, diseases, or proteins that do not regulate but rather are affected by the group of a patient’s genes with similar differential expression patterns. The direction and effect of interactions from the network are used to identify major regulators and major targets. Also, paired nondirectional interactions can be used to find subnetworks of binding partners, affected cells, or coregulated diseases and processes. [Fig. 3](#) depicts examples of SNEA subnetworks: disease or biological processes regulated by proteins with altered expression patterns, binding partners, or expression targets of transcription factors.

Depending on the subnetwork category, an SNEA result can have different biological interpretations. For instance, if the analysis is restricted to “promoter binding” interactions between proteins, then the top-scoring networks reveal transcription factors (“regulators”) that cause significant changes in downstream gene expression levels. If “molecular synthesis” was chosen to explain metabolic omics profiles, the results are interpreted as enzymes that produce the most affected metabolites (all types of relationships for subnetwork combinations are described in “Guide and Legend”).

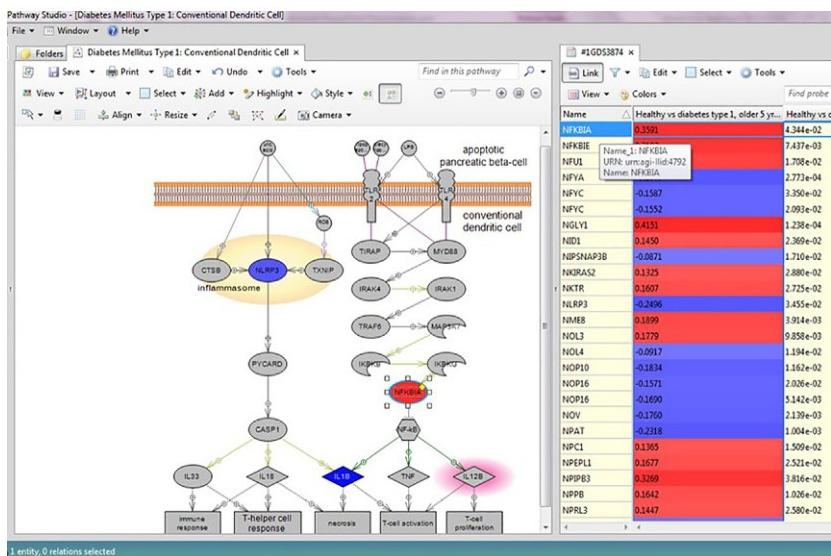


**FIG. 3** Results of SNEA in Pathway Studio. Overexpressed proteins highlighted in red; downexpressed—in blue. Left to right and top to bottom: cellular process subnetwork; cell types subnetwork; transcription factors subnetwork; binding partners subnetwork. For detailed legend see Guide and Legend.

In the last step, GSEA is used to find pathways or GO terms that are enriched with genes from significantly scored subnetworks. For example, the most active or repressed regulators identified by SNEA can be used to find the most active or repressed pathways using GSEA. Gene-set functional enrichment analyses in Pathway Studio also allow for the narrowing down of the analysis with pathways by cell type or tissue because experts annotated the anatomic specificity of each pathway. For example, one can conduct an SNEA of a blood sample only on pathways relevant to the function of different blood cells. So the result would be a list of cells in which activity is higher or lower than normal (the first two steps, SNEA) specifically in blood cell pathways (the third step, GSEA).

Also, it is obvious that one of the quickest ways to use a pathway collection to interpret experimental data is to link the pathway directly to differential gene expression values (Fig. 4). Similar to other applications, Pathway Studio colors the genes on pathways according to their respective values of the differential expression. Unfortunately, this type of analysis “at a glance” can be done meaningfully only if one knows what biological process is expected to be altered by their differential expression.

It is worth summarizing that pathway and network analyses transfer pathway collections and networks from an overview, population level to



**FIG. 4** Visualization of links between patient data (right) and canonical pathway model (left) in Pathway Studio. Overexpressed proteins highlighted in red; downexpressed—in blue. For detailed legend see Guide and Legend.

the level of an individual patient's disease. The set of individual disease networks or pathways may ease the understanding of a patient's status by determining how the personal "disease" pathway differs from typical pathological mechanisms and from the healthy condition. This, for example, can help to select personal biomarkers to test and evaluate for evidence of further disease development. Personal data combined with canonical knowledge can also be used for the selection of rational drug targets optimized for each individual patient. This can be done by defining (1) the key functional pathways, (2) proteins with the largest number of connections on these pathways, (3) major upstream regulators of the patient's differently expressed genes, and (4) major downstream targets that receive common pathway signals. Of course, other algorithms also exist and are needed to find a balance between efficacy, toxicity, and side effects of any chosen therapy. The ideal result of pathway analysis for drug target selection is the identification of selective inhibitors and activators for significant targets in the pathway (Yuryev, 2008).

### In silico modeling in biomedicine

An enrichment analysis based on gene sets, pathways, or networks is used mostly for grouping the molecules identified from high-throughput experiments to understand their function. There is a separate approach

in bioinformatics that does not aim to analyze omics data, but rather, it focuses on the mathematical modeling of the biological processes themselves.

As we mentioned before, mathematical modeling, together with enrichment analyses, is used not only to group genes but also to autonomously reconstruct coexpression clusters and to discover substructures within networks of gene interactions. The mathematical clustering of expressed genes into subnetworks or possible molecular cascades offers unbiased hypotheses about the molecular causes of disease. Although enrichment analysis is more effective for this task, clustering techniques are also important, especially if there are no pathway collections of gene sets available for a given disease. A model based on whole-genome expression data can be used to postulate new genetic interactions and to discover new biomarkers or causative gene candidates (Boucher and Jenna, 2013).

However, the main application of mathematical models is in the examination of the dynamic behavior of the modeled biological process to predict possible real-life response. Rules that describe dependencies between quantitative features are needed to create a dynamic molecular model. Measurements of differential gene expression at different time points, kinetic rate constants, concentrations of proteins, and other parameters can be used as the necessary quantitative features. The use of numbers as special attributes of interactions in the graph allows the conversion of any pathway model into a numeric dynamic model. Moreover, subnetworks and manually reconstructed pathways that are based on directional interactions with known effects (i.e., stimulating and inhibiting) can be used as dynamic models too, because the cascade of interactions can be considered as a set of deterministic (algorithmic) rules that are convertible into mathematical equations.

Models with dynamic or kinetic properties have been used mostly for the simulation and prediction of their behavior and their response to internal changes or external triggers. Therefore, dynamic mathematical models allow testing in silico of a preselected hypothesis and optimization of the design of planned experiment. For example, simulation of the signaling pathway in pancreas beta cells can predict how the cells would respond to treatment with a different dose of insulin (ElKalaawy and Wassal, 2015; Shi et al., 2018; Xiong and Choe, 2008). Quantitative values are also used in the mathematical analysis of models with biological circuits and feedback loops and for metabolic reaction modeling (Alon, 2006).

Disease pathway models supplemented with quantitative features can be useful for many applications in personalized medicine because they can illustrate disease progression over time at the molecular level. This approach can help evaluate a hypothesis about patient's disease dynamics and to improve strategies to further test the hypothesis. However, more

often than not, there is not enough accumulated data to build personal dynamic disease pathways.

Nevertheless, dynamic models are broadly used for analysis not at the level of molecular signaling but at higher levels of complex systems including cells, tissues, and functional organ systems. For example, a dynamic model based on individual patterns of gene expression can be used for examination of the immune system's response against new pathogens or medications before additional laboratory tests are done. Models that rely on simulations of complex system dynamics such as the immune response are often based on the agent-based modeling technique that is one of the popular implementations of the dynamic modeling approach (Broderick, 2012; Chen et al., 2014; Folcik et al., 2011; Walpole et al., 2013).

Agent-based models describe a complex system's collective behavior that emerges from the interaction of autonomous agents (objects) that follow prescribed individual deterministic rules. Agents are supposed to be able to send or respond to signals and can represent any entity of interest such as a molecule, a cell, or a multicellular organism. Rules include any algorithms that describe the signal transfer from one agent to another. Quantitative or stochastic characteristics can also be a part of the rules in agent-based models (Gorochowski, 2016; Somogyi et al., 2016).

Each agent-based model is unique by analogy with manually created individual signaling pathways. This modeling approach may be called synthetic biology because it is aimed to develop "synthetic systems" such as a "bacterial cell models" that mimic the regulation of real biological scenarios. Typically, agent-based models of human biology exploit the collective behavior of well-studied multicellular systems like the immune system, modeling dynamic changes in cell activation, differentiation, and chemotaxis based on existing knowledge about the process and also the molecular profile of each cell (Chiacchio et al., 2014) (more about model types in [Chapter 1](#), "Introduction").

The next notable application of dynamic models intersects with the task of finding cross talk between pathways that is relevant for network-based and pathway topology-based analysis. Estimating dynamic model features including stability, controllability, time-dependent behavior, and robustness is used for grouping models. For example, an unstable system is considered to be sensitive to perturbations or noise that can lead to impairment of the system's functions. That is why the subdivision of models into stable and unstable ones may be used to distinguish hypothetical models of disease, among others. Some authors use the term "dynamic pathway analysis" for finding cross talk between pathways and omics data. For example, Hu and Patterson used dynamic pathway analyses to describe the process of finding pathways or subnetworks associated with whole-genome sequencing data measured at three time points. They used

this approach to try to find communication changes that occur over time between blood pressure-related pathways to describe the steps in blood pressure dynamics (Hu and Paterson, 2014).

Mathematical modeling is generally based on mathematical formulations of differential equations and algorithmic and statistic techniques. Besides the model itself the creation of specific methods for the simulation and calibration should be built. Differential equations are the first-choice methodology of many researchers for the dynamic modeling of biological systems. Some other methods that have been used to create models of biology processes include probabilistic statistics, clustering approaches, machine learning, the graph theory-related methodology of Petri nets and Bayesian networks, binary logic-related Boolean networks, process algebras/calculi family of formal textual languages, a discrete cellular automaton model, and the previously mentioned agent-based modeling (Baker et al., 2018; ElKalaawy and Wassal, 2015).

The modeling of biological processes, especially when based on high-throughput experiments, should integrate both algorithmic and stochastic approaches. By the algorithmic approach, we mean the set of rules by which the deterministic causal hypotheses can be built. For example, specific types of interactions between molecules comprise such rules. Interactions with positive or negative effects are the simplest cases of types of molecular interactions that can serve as an algorithmic rule. Special rules for the cyclic oscillation and dynamic behavior of the modeled process can also be considered. By the stochastic approach, we mean methods that take into account stochastic fluctuations (noise) because a molecular biological process is always influenced by random (statistical) changes.

Since each molecular model is typically created with a unique combination of methods, many tools and software frameworks have been developed to build mathematical models and to analyze them. NetLogo and StarLogo are the most widely used frameworks to create agent-based models. These tools are based on the Logo programming language that allows rules to be defined for the interactions between objects in the model and for running the simulation. Also, there are special tools for modeling multicell behavior (e.g., CompuCell3D, <http://www.compuCell3d.org>).

Several tools (CompuCell3D; Chaste, <http://www.cs.ox.ac.uk/chaste>; and Virtual Cell, <http://vcell.org>) work with the special formats common for pathway models such as systems biology markup language (SBML) and The Synthetic Biology Open Language (SBOL) and thus can ingest third-party models and pathway collections as templates for analysis. Typically the tools and software environments for mathematical modeling are not intended for biologists and require the user to have skill in programming and mathematics (Gorochowski, 2016).

## Tools and repositories for pathway analysis

### Pathway collections and network databases

The efficient interpretation of patient data with enrichment analyses is based on “three pillars”: (1) a comprehensive database with gene sets, molecular interactions, and pathways; (2) powerful analytical methods, and (3) a stable, unified software solution.

First the quality and completeness of the pathway collection are critical requirements for a successful functional enrichment analysis. The more extensive and detailed the collection of pathway models is, the more accurate is the analysis. Moreover, only pathways built for the specific therapeutic area (or even for tissues or cell types that were analyzed in samples) should be used to avoid noise from similar but irrelevant processes that occur in different tissues and diseases within the patient’s dataset. The analysis of some complex diseases may require a vast collection of pathways. For example, the collection of diabetic pathways in Pathway Studio contains more than 140 pathways.

Currently, there are several resources where collections of pathways can be found.

Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome are examples of popular public sources of pathways ([Kanehisa, 2000](#); [Vastrik et al., 2007](#)). KEGG is organized into 530 pathways that include 611,592 references. Reactome has 2256 maps for *Homo sapiens* that cover 10,792 objects. However, most of the pathways there are metabolic pathways and are therefore less cell-specific or disease-specific. The Small Molecule Pathway Database (SMPDB) contains 1451 human-specific proteins, 691 interactions, and claims to contain more than 40,000 “pathways” (<http://smpdb.ca/>).

Commercial pathway collections are considered to be more stable and comprehensive than noncommercial public collections ([Thomas and Bonchev, 2010](#)). The Elsevier Pathway Collection of 2018 contains 2411 pathways and groups covering more than 9000 objects (6351 proteins, 2165 compounds, etc.) and 47,000 relationships. The disease pathways that were reconstructed for this book cover 2368 objects, 5944 relationships, and more than 3000 references to articles, and they can be browsed or downloaded for analysis (<https://mammalcedfx.pathwaystudio.com/app/search>). Quigen (Ingenuity) ([Krämer et al., 2014](#)), Cerelal (MetaCore), and ProteinLounge ([www.proteinlounge.com](http://www.proteinlounge.com)) are other examples of commercial solutions that contain pathway collections.

Although pathways about similar biological functions and even with same names are present in pathway repositories, the diversity of pathway formats and content is the reason why different databases can yield divergent results from the same input experiment. Pathway models are

reconstructed manually by scientists and depend on their subjective interpretation. Therefore, the coverage, content, structure, and functionality of pathway collections vary. Even similarly named pathways can exhibit great differences. Usually, it is very difficult to reproduce the results of pathway enrichment analyses using a different modeling method.

The recommendation here is to use several pathway collections in the analysis or even better select specific and relevant pathways from all database sources. Surely, there were many attempts to aggregate available pathway ontologies in one merged pathway database. Several public databases accumulate information from other available pathway resources; for example, Consensus-PathDB-human integrates 170,276 objects and 603,543 individual interactions from 32 public resources (<http://cpdb.molgen.mpg.de>). Similarly, PathwayCommons (Cerami et al., 2011) seems to be a stable noncommercial resource that aggregates information from 22 resources and currently covers 17,000 gene symbols. PathwayCommons converted all pathways to one universal format, BioPAX. The enumeration of pathway databases can be found in reviews (Ramanan et al., 2012; Tsui et al., 2007) and web resources like Pathguide (<http://www.pathguide.org>). However, it is worth noting that many of the databases described in reviews are not maintained long after their creation and the web links that are supposed to lead to them are often outdated or invalid.

Databases like PathwayCommons that integrate available pathway collections into one network or solutions like Pathway Studio that connect manually reconstructed pathways to the global interactome database are much more powerful instruments for patient enrichment analysis than pathway collections. To get more accurate and precise results, many domain-focused databases should be integrated and used in microarray analyses (Hecker et al., 2009; Tsui et al., 2007). Databases that keep information about genes, proteins, compounds, and diseases are themselves a primary source of annotations. Then, there are databases like GEO or the NCBI that store previously published microarray or sequencing experiment data. These databases are publicly available and are supported by large scientific institutions such as the National Center of Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov>) and the European Bioinformatics Institute (EMBL-EBI, <https://www.ebi.ac.uk/services>). The next database category unites databases that integrate and transform information from core databases. For example, the Human Protein Atlas or Bgee offers associations between anatomical locations and calculated “normal” levels of gene expression. Finally, databases that keep evidence about molecular interactions serve as a foundation for pathway construction and analysis. For example, OMIM (<omim.org>) and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar>) review genetic mutations and all their known associations with human diseases. Today, there are many specialized databases including those that focus on metabolic reactions, physical protein-protein interactions,

transcription factors and gene expression, genetic and epigenetic regulation, or functional interactions between molecules extracted from the literature (see “Links” for the list of useful resources).

Finally, there are databases that combine, in different ways, available molecular interactions into one interactome network. For example, the STRING and BioGRID databases have a global network of protein interactions rather than separate pathways with arbitrarily chosen beginnings and ends. STRING has 18,838 proteins with network connections, and BioGRID has 23,291 for humans. The ResNet molecular interaction database that was used to reconstruct the pathways described in this book covers 93,000 objects and more than 11 million relationships (more about ResNet statistics in “Guide and Legend”).

## Software and tools

The comparability of a pathway collection with tools for data analysis and pathway reconstruction is another important checkpoint for pathway analysis. As we mentioned before, choosing the right application for pathway analysis is not an easy decision. There are too many tools that use different methods and pathway collections, so the results of analyses of the same data made with different tools and pathway collections will likely differ.

Since standards for estimating the results of a patient’s molecular screening are not established yet, all conclusions depend on the level of a doctor’s or biologist’s expertise and their ability to interpret the information. The ideal bioinformatics application for personal medicine should be easy enough to use (at least, in the last steps of the analysis) so the doctor or general biologist can make meaningful conclusions. Also, it should include methods for different types of analyses. Next, many tools or web services for pathway analysis, as with any other applications, may lose maintenance and support from their developers after several months of working. Therefore, stability and “long life” are also important criteria for choosing the right tool for pathway analysis in biomedicine.

There are not many tools that combine all pathway-associated functionalities including search and visualization, reconstruction, prediction, and data analysis. For example, one of the popular features of pathway analysis is the ability to combine pathway visualization with experimental data by coloring genes on pathway images according to their level of differential expression or other measurements. Cytoscape is one of the most frequently used publicly available tools today that can handle all bioinformatics tasks related to pathways, networks, and gene sets (<https://cytoscape.org>).

There are many freeware applications specializing in discrete parts of the process such as pathway visualization, building models de novo, or performing special variations of analyses (Agapito et al., 2013; Fabregat

et al., 2017; García-Campos et al., 2015; Heberle et al., 2017; Hernández-de-Diego et al., 2018; Khatri et al., 2012; Koumakis et al., 2017; Nguyen et al., 2018). The analysis can focus on very particular things like pathway stoichiometry and the steady state of a metabolic network (Faust et al., 2009). PathVisio, which is integrated with the WikiPathways database, is one of the most popular publicly and freely available software packages for pathway reconstruction, visualization, and some types of pathway analysis (<https://www.pathvisio.org>) (Kutmon et al., 2015). DAVID is a favorite tool for performing ORA (Huang et al., 2009).

Usually, free open-source tools for pathway analysis are supplemented with separate statistical packages and require the ability to code in R, Python, or other programming languages (Gentleman et al., 2004; Henderson-Maclennan et al., 2010). This requires special skills that are not yet common among biologists.

Commercial applications are usually easy to use, and they combine everything in a single software package including pathway collections, analysis algorithms, and tools for pathway reconstruction. Elsevier Pathway Studio (Elsevier, <http://www.pathwaystudio.com>), Ingenuity Pathways Analysis (<https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>) and BioBase/ExPlain (now both are under Qiagen), the Genomatix Software Suit (Genomatix, <https://www.genomatix.de>), MetaCore (Cortellis), iPathwayGuide (Advaita Corporation, <https://www.advaitabio.com>), and others offer their solutions, specializing in different aspects of pathway analysis (Bogner et al., 2011; Henderson-Maclennan et al., 2010; Nguyen et al., 2018; Thomas and Bonchev, 2010; Werner, 2008; Yuryev, 2011). The differences between different commercial applications lie in their methods of database reconstruction, the amount of collected data, the number of techniques included, and the overall design of their analytic approach. Therefore, it is beneficial to combine software tools and resources to attain the most complete analysis of patient data.

## Conclusion

Research of disease mechanisms has a long history. Just recently, scientists began searching for disease origins at the level of molecular interactions. Moreover, it was not long ago that we learned that the lack of a single protein or a mutation in a single gene can cause serious and complex illnesses. However, most diseases appear to involve malfunctions in multiple proteins and genes that in turn lead to pathological processes at higher organizational levels of cells and tissues. It became clear that studying disease manifestations at the molecular, cellular, and physiological levels means processing huge amounts of information to separate primary aspects of the pathology from auxiliary ones.

During the short period since molecular methods have been introduced into medical research and because of the extensive development of computer technologies, a great amount of experimental data have accumulated. In addition, our capabilities for examining the function of all genes simultaneously in a given patient are expanding. Huge data arrays are most likely keeping the key to understanding the pathogenesis of one or another disease, but how do we leverage these data to cut this Gordian knot?

The application of math and informatics is combining as much as knowledge possible about genes, proteins, drugs, and diseases into a single network, the interactome. Graph databases and networks turn out to be “a natural way to represent highly connected, nonuniformly distributed, semistructured, and unpredictable data as found in many biological systems studies” (Lysenko et al., 2016). The network-integrated experimental data about the functions and behaviors of biologically active molecules are meant to help biologists generate assumptions and hypotheses.

Molecular interactions between proteins are the basis for an artificial interactome and disease models derived from it. In this book, we described in detail human disease signaling pathway models and the prevalent application of molecular interaction models in the analysis of individual gene expression profiles as a solid base for precision medicine.

Networks and pathways have been shown to have high predictive accuracy for the identification of individual disease-causing genes and disease biomarkers. They have also been used to classify subtypes or the development stages of a disease. Finally, network pharmacology is becoming increasingly important to predict effective new drug targets.

Pathway and network analyses are still the methods that cannot be widely used in medical practice. Most conclusions drawn from these analyses are based on statistical regularities and must be interpreted with care. However, we hope that the potential strength of disease signaling pathways will be applied to diagnostics and individual therapy selection, just as has happened with identified gene variants in pharmacogenomics. The emergence of integrated data resources and convenient and easy software will further contribute to this goal.

## References

- Acharya, S., Saha, S., Pradhan, P., 2018. Novel symmetry-based gene-gene dissimilarity measures utilizing Gene Ontology: application in gene clustering. *Gene* 679, 341–351. <https://doi.org/10.1016/j.gene.2018.08.062>.
- Ackermann, M., Strimmer, K., 2009. A general modular framework for gene set enrichment analysis. *BMC Bioinformatics* 10, 47. <https://doi.org/10.1186/1471-2105-10-47>.
- Agapito, G., Guzzi, P.H., Cannataro, M., 2013. Visualization of protein interaction networks: problems and solutions. *BMC Bioinformatics* 14, S1. <https://doi.org/10.1186/1471-2105-14-S1-S1>.

- Alaimo, S., Marcea, G.P., Ferro, A., Pulvirenti, A., 2017. Detecting disease specific pathway substructures through an integrated systems biology approach. *Non-Coding RNA* 3. <https://doi.org/10.3390/ncrna3020020>.
- Alexeyenko, A., Lee, W., Pernemalm, M., Guegan, J., Dessen, P., Lazar, V., Lehtiö, J., Pawitan, Y., 2012. Network enrichment analysis: extension of gene-set enrichment analysis to gene networks. *BMC Bioinformatics* 13, 226. <https://doi.org/10.1186/1471-2105-13-226>.
- Allison, D.B., Cui, X., Page, G.P., Sabripour, M., 2006. Microarray data analysis: from disarray to consolidation and consensus. *Nat. Rev. Genet.* 7, 55–65. <https://doi.org/10.1038/nrg1749>.
- Alon, U., 2006. *An Introduction to Systems Biology: Design Principles of Biological Circuits*. CRC Press Book, Chapman & Hall/CRC Mathematical and Computational Biology. Chapman and Hall/CRC.
- Alyass, A., Turcotte, M., Meyre, D., 2015. From big data analysis to personalized medicine for all: challenges and opportunities. *BMC Med. Genet.* 8. <https://doi.org/10.1186/s12920-015-0108-y>.
- Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., Harris, M.A., Hill, D.P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J.C., Richardson, J.E., Ringwald, M., Rubin, G.M., Sherlock, G., 2000. Gene Ontology: tool for the unification of biology. *Nat. Genet.* 25, 25–29. <https://doi.org/10.1038/75556>.
- Aslibekyan, S., Almeida, M., Tintle, N., 2014. Pathway analysis approaches for rare and common variants: insights from Genetic Analysis Workshop 18. *Genet. Epidemiol.* 38 (Suppl. 1), S86–S91. <https://doi.org/10.1002/gepi.21831>.
- Ay, A., Arnosti, D.N., 2011. Mathematical modeling of gene expression: a guide for the perplexed biologist. *Crit. Rev. Biochem. Mol. Biol.* 46, 137–151. <https://doi.org/10.3109/10409238.2011.556597>.
- Baker, R.E., Peña, J.-M., Jayamohan, J., Jérusalem, A., 2018. Mechanistic models versus machine learning, a fight worth fighting for the biological community? *Biol. Lett.* 14, 20170660. <https://doi.org/10.1098/rsbl.2017.0660>.
- Bal-Price, A., Lein, P.J., Keil, K.P., Sethi, S., Shafer, T., Barenys, M., Fritzsche, E., Sachana, M., Meek, M.E.B., 2017. Developing and applying the adverse outcome pathway concept for understanding and predicting neurotoxicity. *Neurotoxicology* 59, 240–255. <https://doi.org/10.1016/j.neuro.2016.05.010>.
- Barbosa, S., Niebel, B., Wolf, S., Mauch, K., Takors, R., 2018. A guide to gene regulatory network inference for obtaining predictive solutions: underlying assumptions and fundamental biological and data constraints. *Biosystems* 174, 37–48. <https://doi.org/10.1016/j.biosystems.2018.10.008>.
- Bogner, V., Leidel, B.A., Kanz, K.-G., Mutschler, W., Neugebauer, E.A.M., Biberthaler, P., 2011. Pathway analysis in microarray data: a comparison of two different pathway analysis devices in the same data set. *Shock* 35, 245–251. <https://doi.org/10.1097/SHK.0b013e3181fc904d>.
- Boucher, B., Jenna, S., 2013. Genetic interaction networks: better understand to better predict. *Front. Genet.* 4, <https://doi.org/10.3389/fgene.2013.00290>.
- Braun, R., Shah, S., 2014. Network Methods for Pathway Analysis of Genomic Data. ArXiv14111993 Q-Bio Stat.
- Broderick, G., 2012. A moving target: taking aim at the regulatory dynamics of illness. *Brain Behav. Immun.* 26, 1045–1046. <https://doi.org/10.1016/j.bbi.2012.06.013>.
- Cerami, E.G., Gross, B.E., Demir, E., Rodchenkov, I., Babur, Ö., Anwar, N., Schultz, N., Bader, G.D., Sander, C., 2011. Pathway Commons, a web resource for biological pathway data. *Nucleic Acids Res.* 39, D685–D690. <https://doi.org/10.1093/nar/gkq1039>.
- Cheadle, C., Cao, H., Kalinin, A., Hodgkinson, J., 2017. Advanced literature analysis in a Big Data world. *Ann. N. Y. Acad. Sci.* 1387, 25–33. <https://doi.org/10.1111/nyas.13270>.

- Chen, X., Hickling, T.P., Vicini, P., 2014. A mechanistic, multiscale mathematical model of immunogenicity for therapeutic proteins: Part 1—Theoretical model. *CPT Pharmacometrics Syst. Pharmacol.* 3, e133. <https://doi.org/10.1038/psp.2014.30>.
- Chiacchio, F., Pennisi, M., Russo, G., Motta, S., Pappalardo, F., 2014. Agent-based modeling of the immune system: NetLogo, a promising framework. *Biomed. Res. Int.* 2014. <https://doi.org/10.1155/2014/907171>.
- Copeland, W.B., Bartley, B.A., Chandran, D., Galdzicki, M., Kim, K.H., Sleight, S.C., Maranas, C.D., Sauro, H.M., 2012. Computational tools for metabolic engineering. *Metab. Eng.* 14, 270–280. <https://doi.org/10.1016/j.ymben.2012.03.001>.
- Donato, M., Xu, Z., Tomoiaga, A., Granneman, J.G., Mackenzie, R.G., Bao, R., Than, N.G., Westfall, P.H., Romero, R., Draghici, S., 2013. Analysis and correction of crosstalk effects in pathway analysis. *Genome Res.* 23, 1885–1893. <https://doi.org/10.1101/gr.153551.112>.
- Dong, X., Hao, Y., Wang, X., Tian, W., 2016. LEGO: a novel method for gene set overrepresentation analysis by incorporating network-based gene weights. *Sci. Rep.* 6, 18871. <https://doi.org/10.1038/srep18871>.
- Dutta, B., Wallqvist, A., Reifman, J., 2012. PathNet: a tool for pathway analysis using topological information. *Source Code Biol. Med.* 7, 10. <https://doi.org/10.1186/1751-0473-7-10>.
- Egorov, S., Yuryev, A., Daraselia, N., 2004. A simple and practical dictionary-based approach for identification of proteins in Medline abstracts. *J. Am. Med. Inform. Assoc.* 11, 174–178. <https://doi.org/10.1197/jamia.M1453>.
- ElKalaawy, N., Wassal, A., 2015. Methodologies for the modeling and simulation of biochemical networks, illustrated for signal transduction pathways: a primer. *Biosystems* 129, 1–18. <https://doi.org/10.1016/j.biosystems.2015.01.008>.
- Emmert-Streib, F., Glazko, G.V., 2011. Pathway analysis of expression data: deciphering functional building blocks of complex diseases. *PLoS Comput. Biol.* 7, e1002053. <https://doi.org/10.1371/journal.pcbi.1002053>.
- Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V., D'Eustachio, P., Stein, L., Hermjakob, H., 2017. Reactome pathway analysis: a high-performance in-memory approach. *BMC Bioinformatics* 18, 142. <https://doi.org/10.1186/s12859-017-1559-2>.
- Faust, K., Croes, D., van Helden, J., 2009. In response to “Can sugars be produced from fatty acids? A test case for pathway analysis tools”. *Bioinformatics* 25, 3202–3205. <https://doi.org/10.1093/bioinformatics/btp557>.
- Folcik, V.A., Broderick, G., Mohan, S., Block, B., Ekbote, C., Doolittle, J., Khoury, M., Davis, L., Marsh, C.B., 2011. Using an agent-based model to analyze the dynamic communication network of the immune response. *Theor. Biol. Med. Model.* 8, 1. <https://doi.org/10.1186/1742-4682-8-1>.
- García-Campos, M.A., Espinal-Enríquez, J., Hernández-Lemus, E., 2015. Pathway analysis: state of the art. *Front. Physiol.* 6, <https://doi.org/10.3389/fphys.2015.00383>.
- Gene Ontology Consortium, 2017. Expansion of the Gene Ontology knowledgebase and resources. *Nucleic Acids Res.* 4, D331–D338. <https://doi.org/10.1093/nar/gkw1108>.
- Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y.H., Zhang, J., 2004. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 5, R80. <https://doi.org/10.1186/gb-2004-5-10-r80>.
- Glaab, E., Baudot, A., Krasnogor, N., Schneider, R., Valencia, A., 2012. EnrichNet: network-based gene set enrichment analysis. *Bioinformatics* 28, i451–i457. <https://doi.org/10.1093/bioinformatics/bts389>.
- Glazko, G.V., Emmert-Streib, F., 2009. Unite and conquer: univariate and multivariate approaches for finding differentially expressed gene sets. *Bioinformatics* 25, 2348–2354. <https://doi.org/10.1093/bioinformatics/btp406>.

- Gligorijević, V., Malod-Dognin, N., Pržulj, N., 2016. Integrative methods for analyzing big data in precision medicine. *Proteomics* 16, 741–758. <https://doi.org/10.1002/pmic.201500396>.
- Goeman, J.J., Bühlmann, P., 2007. Analyzing gene expression data in terms of gene sets: methodological issues. *Bioinformatics* 23, 980–987. <https://doi.org/10.1093/bioinformatics/btm051>.
- Gorochowski, T.E., 2016. Agent-based modelling in synthetic biology. *Essays Biochem.* 60, 325–336. <https://doi.org/10.1042/EBC20160037>.
- Haber, D.A., Settleman, J., 2007. Cancer: drivers and passengers. *Nature* 446, 145–146. <https://doi.org/10.1038/446145a>.
- Hao, T., Wang, Q., Zhao, L., Wu, D., Wang, E., Sun, J., 2018. Analyzing of molecular networks for human diseases and drug discovery. *Curr. Top. Med. Chem.* 18, 1007–1014. <https://doi.org/10.2174/1568026618666180813143408>.
- Haynes, L.D., Jankowska-Gan, E., Sheka, A., Keller, M.R., Hernandez-Fuentes, M.P., Lechler, R.I., Seyfert-Margolis, V., Turka, L.A., Newell, K.A., Burlingham, W.J., 2012. Donor-specific indirect pathway analysis reveals a B-cell-independent signature which reflects outcomes in kidney transplant recipients. *Am. J. Transplant. Off. J. Am. Soc. Transplant. Am. Soc. Transpl. Surg.* 12, 640–648. <https://doi.org/10.1111/j.1600-6143.2011.03869.x>.
- He, K.Y., Ge, D., He, M.M., 2017. Big data analytics for genomic medicine. *Int. J. Mol. Sci.* 18, <https://doi.org/10.3390/ijms18020412>.
- Heberle, H., Carazzolle, M.F., Telles, G.P., Meirelles, G.V., Minghim, R., 2017. CellNetVis: a web tool for visualization of biological networks using force-directed layout constrained by cellular components. *BMC Bioinformatics* 18, 395. <https://doi.org/10.1186/s12859-017-1787-5>.
- Hecker, M., Lambeck, S., Toepfer, S., van Someren, E., Guthke, R., 2009. Gene regulatory network inference: data integration in dynamic models—a review. *Biosystems* 96, 86–103. <https://doi.org/10.1016/j.biosystems.2008.12.004>.
- Hedlund, E., Deng, Q., 2018. Single-cell RNA sequencing: technical advancements and biological applications. *Mol. Aspects Med., The emerging field of single-cell analysis* 59, 36–46. <https://doi.org/10.1016/j.mam.2017.07.003>.
- Henderson-Maclennan, N.K., Papp, J.C., Talbot, C.C., McCabe, E.R.B., Presson, A.P., 2010. Pathway analysis software: annotation errors and solutions. *Mol. Genet. Metab.* 101, 134–140. <https://doi.org/10.1016/j.ymgme.2010.06.005>.
- Hernández-de-Diego, R., Tarazona, S., Martínez-Mira, C., Balzano-Nogueira, L., Furió-Tarí, P., Pappas, G.J., Conesa, A., 2018. PaintOmics 3: a web resource for the pathway analysis and visualization of multi-omics data. *Nucleic Acids Res.* 46, W503–W509. <https://doi.org/10.1093/nar/gky466>.
- Himmelstein, D.S., Lizze, A., Hessler, C., Brueggeman, L., Chen, S.L., Hadley, D., Green, A., Khankhanian, P., Baranzini, S.E., 2017. Systematic integration of biomedical knowledge prioritizes drugs for repurposing. *eLife*. <https://doi.org/10.7554/eLife.26726>.
- Holmans, P., 2010. Statistical methods for pathway analysis of genome-wide data for association with complex genetic traits. *Adv. Genet.* 72, 141–179. <https://doi.org/10.1016/B978-0-12-380862-2.00007-2>.
- Houlston, R.S., Tomlinson, I.P., 1998. Modifier genes in humans: strategies for identification. *Eur. J. Hum. Genet.* 6, 80–88. <https://doi.org/10.1038/sj.ejhg.5200156>.
- Hu, P., Paterson, A.D., 2014. Dynamic pathway analysis of genes associated with blood pressure using whole genome sequence data. *BMC Proc.* 8, S106. <https://doi.org/10.1186/1753-6561-8-S1-S106>.
- Huang, D.W., Sherman, B.T., Lempicki, R.A., 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4, 44–57. <https://doi.org/10.1038/nprot.2008.211>.

- Hung, J.-H., Yang, T.-H., Hu, Z., Weng, Z., DeLisi, C., 2012. Gene set enrichment analysis: performance evaluation and usage guidelines. *Brief. Bioinform.* 13, 281–291. <https://doi.org/10.1093/bib/bbr049>.
- Ihnatova, I., Popovici, V., Budinska, E., 2018. A critical comparison of topology-based pathway analysis methods. *PLoS One* 13, e0191154. <https://doi.org/10.1371/journal.pone.0191154>.
- Kanehisa, M., 2000. Post-Genome Informatics. Oxford University Press, Oxford, New York.
- Kao, P.Y.P., Leung, K.H., Chan, L.W.C., Yip, S.P., Yap, M.K.H., 2017. Pathway analysis of complex diseases for GWAS, extending to consider rare variants, multi-omics and interactions. *Biochim. Biophys. Acta Gen. Subj.* 1861, 335–353. <https://doi.org/10.1016/j.bbagen.2016.11.030>.
- Kavanagh, T., Mills, J.D., Kim, W.S., Halliday, G.M., Janitz, M., 2013. Pathway analysis of the human brain transcriptome in disease. *J. Mol. Neurosci.* 51, 28–36. <https://doi.org/10.1007/s12031-012-9940-0>.
- Khatri, P., Drăghici, S., 2005. Ontological analysis of gene expression data: current tools, limitations, and open problems. *Bioinformatics* 21, 3587–3595. <https://doi.org/10.1093/bioinformatics/bti565>.
- Khatri, P., Sirota, M., Butte, A.J., 2012. Ten years of pathway analysis: current approaches and outstanding challenges. *PLoS Comput. Biol.* 8, e1002375. <https://doi.org/10.1371/journal.pcbi.1002375>.
- Kim, T.Y., Kim, H.U., Lee, S.Y., 2010. Data integration and analysis of biological networks. *Curr. Opin. Biotechnol., Analytical Biotechnology* 21, 78–84. <https://doi.org/10.1016/j.copbio.2010.01.003>.
- Kontou, P.I., Pavlopoulou, A., Dimou, N.L., Pavlopoulos, G.A., Bagos, P.G., 2016. Network analysis of genes and their association with diseases. *Gene* 590, 68–78. <https://doi.org/10.1016/j.gene.2016.05.044>.
- Kotelnikova, E.A., Pyatnitskiy, M., Paleeva, A., Kremenetskaya, O., Vinogradov, D., 2016. Practical aspects of NGS-based pathways analysis for personalized cancer science and medicine. *Oncotarget* 7, 52493–52516. <https://doi.org/10.18632/oncotarget.9370>.
- Koumakis, L., Roussos, P., Potamias, G., 2017. minepath.org: a free interactive pathway analysis web server. *Nucleic Acids Res.* 45, W116–W121. <https://doi.org/10.1093/nar/gkx278>.
- Krämer, A., Green, J., Pollard, J., Tugendreich, S., 2014. Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics* 30, 523–530. <https://doi.org/10.1093/bioinformatics/btt703>.
- Kutmon, M., van Iersel, M.P., Bohler, A., Kelder, T., Nunes, N., Pico, A.R., Evelo, C.T., 2015. PathVisio 3: an extendable pathway analysis toolbox. *PLoS Comput. Biol.* 11, e1004085. <https://doi.org/10.1371/journal.pcbi.1004085>.
- Lebeda, F.J., Dembek, Z.F., Adler, M., 2012. Kinetic and reaction pathway analysis in the application of botulinum toxin a for wound healing. *J. Toxicol.* 2012, 159726. <https://doi.org/10.1155/2012/159726>.
- Lehner, B., 2007. Modelling genotype–phenotype relationships and human disease with genetic interaction networks. *J. Exp. Biol.* 210, 1559–1566. <https://doi.org/10.1242/jeb.002311>.
- Lin, S.-J., Lu, T.-P., Yu, Q.-Y., Hsiao, C.K., 2018. Probabilistic prioritization of candidate pathway association with pathway score. *BMC Bioinformatics* 19, 391. <https://doi.org/10.1186/s12859-018-2411-z>.
- Liu, Q., Dinu, I., Adewale, A.J., Potter, J.D., Yasui, Y., 2007a. Comparative evaluation of gene-set analysis methods. *BMC Bioinformatics* 8, 431. <https://doi.org/10.1186/1471-2105-8-431>.
- Liu, M., Liberzon, A., Kong, S.W., Lai, W.R., Park, P.J., Kohane, I.S., Kasif, S., 2007b. Network-based analysis of affected biological processes in type 2 diabetes models. *PLoS Genet.* 3, e96. <https://doi.org/10.1371/journal.pgen.0030096>.

- Liu, Z.-P., Wu, C., Miao, H., Wu, H., 2015. RegNetwork: an integrated database of transcriptional and post-transcriptional regulatory networks in human and mouse. *Database J. Biol. Databases Curation* 2015. <https://doi.org/10.1093/database/bav095>.
- Liu, L., Wei, J., Ruan, J., 2017. Pathway enrichment analysis with networks. *Genes* 8. <https://doi.org/10.3390/genes8100246>.
- Lysenko, A., Roznová, I.A., Saqi, M., Mazein, A., Rawlings, C.J., Auffray, C., 2016. Representing and querying disease networks using graph databases. *BioData Min.* 9, 23. <https://doi.org/10.1186/s13040-016-0102-8>.
- Maciejewski, H., 2014. Gene set analysis methods: statistical models and methodological differences. *Brief. Bioinform.* 15, 504–518. <https://doi.org/10.1093/bib/bbt002>.
- Manoli, T., Gretz, N., Gröne, H.-J., Kenzelmann, M., Eils, R., Brors, B., 2006. Group testing for pathway analysis improves comparability of different microarray datasets. *Bioinformatics* 22, 2500–2506. <https://doi.org/10.1093/bioinformatics/btl424>.
- Mathur, R., Rotroff, D., Ma, J., Shojaie, A., Motsinger-Reif, A., 2018. Gene set analysis methods: a systematic comparison. *BioData Min.* 11, 8. <https://doi.org/10.1186/s13040-018-0166-8>.
- McCormack, T., Frings, O., Alexeyenko, A., Sonnhammer, E.L.L., 2013. Statistical assessment of crosstalk enrichment between gene groups in biological networks. *PLoS One* 8, e54945. <https://doi.org/10.1371/journal.pone.0054945>.
- McCue, M.E., McCoy, A.M., 2017. The scope of big data in one medicine: unprecedented opportunities and challenges. *Front. Vet. Sci.* 4, 194. <https://doi.org/10.3389/fvets.2017.00194>.
- Mejía-Pedroza, R.A., Espinal-Enríquez, J., Hernández-Lemus, E., 2018. Pathway-based drug repositioning for breast cancer molecular subtypes. *Front. Pharmacol.* 9. <https://doi.org/10.3389/fphar.2018.00905>.
- Mieczkowski, J., Swiatek-Machado, K., Kaminska, B., 2012. Identification of pathway deregulation—gene expression based analysis of consistent signal transduction. *PLoS One* 7, e41541. <https://doi.org/10.1371/journal.pone.0041541>.
- Mitre, C., Taghavi, Z., Bokanizad, B., Hanoudi, S., Tagett, R., Donato, M., Voichiță, C., Drăghici, S., 2013. Methods and approaches in the topology-based analysis of biological pathways. *Front. Physiol.* 4, 278. <https://doi.org/10.3389/fphys.2013.00278>.
- Mooney, M.A., Nigg, J.T., McWeney, S.K., Wilmot, B., 2014. Functional and genomic context in pathway analysis of GWAS data. *Trends Genet.* 30, 390–400. <https://doi.org/10.1016/j.tig.2014.07.004>.
- Mootha, V.K., Lindgren, C.M., Eriksson, K.-F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., Houstis, N., Daly, M.J., Patterson, N., Mesirov, J.P., Golub, T.R., Tamayo, P., Spiegelman, B., Lander, E.S., Hirschhorn, J.N., Altshuler, D., Groop, L.C., 2003. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* 34, 267–273. <https://doi.org/10.1038/ng1180>.
- Muñoz Garcia, A., Kutmon, M., Eijssen, L., Hewison, M., Evelo, C.T., Coort, S.L., 2018. Pathway analysis of transcriptomic data shows immunometabolic effects of vitamin D. *J. Mol. Endocrinol.* 60, 95–108. <https://doi.org/10.1530/JME-17-0186>.
- Nam, D., Kim, S.-Y., 2008. Gene-set approach for expression pattern analysis. *Brief. Bioinform.* 9, 189–197. <https://doi.org/10.1093/bib/bbn001>.
- National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease, 2011. Toward Precision Medicine: Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease. The National Academies Collection: Reports funded by National Institutes of Health. National Academies Press (US), Washington, DC.
- Nguyen, T., Mitrea, C., Draghici, S., 2018. Network-based approaches for pathway level analysis. *Curr. Protoc. Bioinformatics* 61, 8.25.1–8.25.24. <https://doi.org/10.1002/cpb1.42>.

- Ogris, C., Guala, D., Helleday, T., Sonnhammer, E.L.L., 2017. A novel method for crosstalk analysis of biological networks: improving accuracy of pathway annotation. *Nucleic Acids Res.* 45, e8. <https://doi.org/10.1093/nar/gkw849>.
- Okada, Y., Sahara, T., Mitsubayashi, H., Ohgiya, S., Nagashima, T., 2005. Knowledge-assisted recognition of cluster boundaries in gene expression data. *Artif. Intell. Med.* 35, 171–183. <https://doi.org/10.1016/j.artmed.2005.02.007>.
- Oyelade, J., Isewon, I., Oladipupo, F., Aromolaran, O., Uwoghiren, E., Ameh, F., Achas, M., Adebiyi, E., 2016. Clustering algorithms: their application to gene expression data. *Bioinform. Biol. Insights* 10, 237–253. <https://doi.org/10.4137/BBI.S38316>.
- Ozerov, I.V., Lezhnina, K.V., Izumchenko, E., Artemov, A.V., Medintsev, S., Vanhaelen, Q., Aliper, A., Vijg, J., Osipov, A.N., Labat, I., West, M.D., Buzdin, A., Cantor, C.R., Nikolsky, Y., Borisov, N., Irincheeva, I., Khokhlovich, E., Sidransky, D., Camargo, M.L., Zhavoronkov, A., 2016. In silico pathway activation network decomposition analysis (iPANDA) as a method for biomarker development. *Nat. Commun.* 7, 13427. <https://doi.org/10.1038/ncomms13427>.
- Paul, K.A., Shill, P.C., 2018. Incorporating gene ontology into fuzzy relational clustering of microarray gene expression data. *Biosystems* 163, 1–10. <https://doi.org/10.1016/j.biosystems.2017.09.017>.
- Pavlidis, P., Qin, J., Arango, V., Mann, J.J., Sibille, E., 2004. Using the gene ontology for microarray data mining: a comparison of methods and application to age effects in human prefrontal cortex. *Neurochem. Res.* 29, 1213–1222. <https://doi.org/10.1023/B:NERE.0000023608.29741.45>.
- Peng, J., Wang, Y., Chen, J., 2014. Towards integrative gene functional similarity measurement. *BMC Bioinformatics* 15, S5. <https://doi.org/10.1186/1471-2105-15-S2-S5>.
- Peterson, T.A., Doughty, E., Kann, M.G., 2013. Towards precision medicine: advances in computational approaches for the analysis of human variants. *J. Mol. Biol.* 425, 4047–4063. <https://doi.org/10.1016/j.jmb.2013.08.008>.
- Pita-Juárez, Y., Altschuler, G., Kariotis, S., Wei, W., Koler, K., Green, C., Tanzi, R.E., Hide, W., 2018. The Pathway Coexpression Network: revealing pathway relationships. *PLoS Comput. Biol.* 14, e1006042. <https://doi.org/10.1371/journal.pcbi.1006042>.
- Pyatnitskiy, M., Mazo, I., Shkrob, M., Schwartz, E., Kotelnikova, E., 2014. Clustering gene expression regulators: new approach to disease subtyping. *PLoS One* 9, e84955. <https://doi.org/10.1371/journal.pone.0084955>.
- Ramanan, V.K., Shen, L., Moore, J.H., Saykin, A.J., 2012. Pathway analysis of genomic data: concepts, methods, and prospects for future development. *Trends Genet.* 28, 323–332. <https://doi.org/10.1016/j.tig.2012.03.004>.
- Rivals, I., Personnaz, L., Taing, L., Potier, M.-C., 2007. Enrichment or depletion of a GO category within a class of genes: which test? *Bioinformatics* 23, 401–407. <https://doi.org/10.1093/bioinformatics/btl633>.
- Seifert, M., Scherf, M., Epple, A., Werner, T., 2005. Multievidence microarray mining. *Trends Genet.* 21, 553–558. <https://doi.org/10.1016/j.tig.2005.07.011>.
- Shi, M., Chong, Y., Shen, W., Xie, X.-P., Wang, H.-Q., 2018. DynSig: modelling dynamic signaling alterations along gene pathways for identifying differential pathways. *Genes* 9. <https://doi.org/10.3390/genes9070323>.
- Shojaie, A., Michailidis, G., 2010. Network enrichment analysis in complex experiments. *Stat. Appl. Genet. Mol. Biol.* 9. <https://doi.org/10.2202/1544-6115.1483>.
- Signorelli, M., Vinciotti, V., Wit, E.C., 2016. NEAT: an efficient network enrichment analysis test. *BMC Bioinformatics* 17, 352. <https://doi.org/10.1186/s12859-016-1203-6>.
- Sivachenko, A.Y., Yuryev, A., 2007. Pathway analysis software as a tool for drug target selection, prioritization and validation of drug mechanism. *Expert Opin. Ther. Targets* 11, 411–421. <https://doi.org/10.1517/14728222.11.3.411>.
- Smyth, G.K., 2004. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* 3, 3. <https://doi.org/10.2202/1544-6115.1027>.

- Somogyi, E., Sluka, J.P., Glazier, J.A., 2016. Formalizing knowledge in multi-scale agent-based simulations. In: Model Driven Eng. Lang. Syst. Int. Conf. MoDELS Proc. MODELS Conf. 16, pp. 115–122. <https://doi.org/10.1145/2976767.2976790>.
- Soneson, C., Delorenzi, M., 2013. A comparison of methods for differential expression analysis of RNA-seq data. *BMC Bioinformatics* 14, 91. <https://doi.org/10.1186/1471-2105-14-91>.
- Spinelli, L., Carpentier, S., Montañana Sanchis, F., Dalod, M., Vu Manh, T.-P., 2015. BubbleGUM: automatic extraction of phenotype molecular signatures and comprehensive visualization of multiple Gene Set Enrichment Analyses. *BMC Genomics* 16, 814. <https://doi.org/10.1186/s12864-015-2012-4>.
- Stratton, M.R., Campbell, P.J., Futreal, P.A., 2009. The cancer genome. *Nature* 458, 719–724. <https://doi.org/10.1038/nature07943>.
- Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., Mesirov, J.P., 2005. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci.* 102, 15545–15550. <https://doi.org/10.1073/pnas.0506580102>.
- Sun, D., Liu, Y., Zhang, X.-S., Wu, L.-Y., 2017. NetGen: a novel network-based probabilistic generative model for gene set functional enrichment analysis. *BMC Syst. Biol.* 11, 75. <https://doi.org/10.1186/s12918-017-0456-7>.
- Sun, D., Liu, Y., Zhang, X.-S., Wu, L.-Y., 2018. CEA: combination-based gene set functional enrichment analysis. *Sci. Rep.* 8, 13085. <https://doi.org/10.1038/s41598-018-31396-4>.
- Tarca, A.L., Romero, R., Draghici, S., 2006. Analysis of microarray experiments of gene expression profiling. *Am. J. Obstet. Gynecol.* 195, 373–388. <https://doi.org/10.1016/j.ajog.2006.07.001>.
- Tarca, A.L., Draghici, S., Khatri, P., Hassan, S.S., Mittal, P., Kim, J., Kim, C.J., Kusanovic, J.P., Romero, R., 2009. A novel signaling pathway impact analysis. *Bioinformatics* 25, 75–82. <https://doi.org/10.1093/bioinformatics/btn577>.
- Thomas, S., Bonchev, D., 2010. A survey of current software for network analysis in molecular biology. *Hum. Genomics* 4, 353. <https://doi.org/10.1186/1479-7364-4-5-353>.
- Tian, L., Greenberg, S.A., Kong, S.W., Altschuler, J., Kohane, I.S., Park, P.J., 2005. Discovering statistically significant pathways in expression profiling studies. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13544–13549. <https://doi.org/10.1073/pnas.0506577102>.
- Tremaine, L., Brian, W., DelMonte, T., Francke, S., Groenen, P., Johnson, K., Li, L., Pearson, K., Marshall, J.-C., 2015. The role of ADME pharmacogenomics in early clinical trials: perspective of the Industry Pharmacogenomics Working Group (I-PWG). *Pharmacogenomics* 16, 2055–2067. <https://doi.org/10.2217/pgs.15.141>.
- Tsui, I.F.L., Chari, R., Buys, T.P.H., Lam, W.L., 2007. Public databases and software for the pathway analysis of cancer genomes. *Cancer Informat.* 3, 117693510700300030. <https://doi.org/10.1177/117693510700300027>.
- Vastrik, I., D'Eustachio, P., Schmidt, E., Joshi-Tope, G., Gopinath, G., Croft, D., de Bono, B., Gillespie, M., Jassal, B., Lewis, S., Matthews, L., Wu, G., Birney, E., Stein, L., 2007. Reactome: a knowledge base of biologic pathways and processes. *Genome Biol.* 8, R39. <https://doi.org/10.1186/gb-2007-8-3-r39>.
- Vella, D., Zoppis, I., Mauri, G., Mauri, P., Di Silvestre, D., 2017. From protein-protein interactions to protein co-expression networks: a new perspective to evaluate large-scale proteomic data. *EURASIP J. Bioinforma. Syst. Biol.* 2017, 6. <https://doi.org/10.1186/s13637-017-0059-z>.
- Walpole, J., Papin, J.A., Peirce, S.M., 2013. Multiscale computational models of complex biological systems. *Annu. Rev. Biomed. Eng.* 15, 137–154. <https://doi.org/10.1146/annurev-bioeng-071811-150104>.
- Wang, Y., Thilimony, R., Gu, Y.Q., 2014. NetVenn: an integrated network analysis web platform for gene lists. *Nucleic Acids Res.* 42, W161–W166. <https://doi.org/10.1093/nar/gku331>.

- Wang, H., Sun, Q., Zhao, W., Qi, L., Gu, Y., Li, P., Zhang, M., Li, Y., Liu, S.-L., Guo, Z., 2015. Individual-level analysis of differential expression of genes and pathways for personalized medicine. *Bioinformatics* 31, 62–68. <https://doi.org/10.1093/bioinformatics/btu522>.
- Wang, Y.-Y., Wang, Z.-X., Hu, Y., Wang, L., Li, N., Zhang, B., Han, W., Jiang, J.-M., 2017. Current status of pathway analysis in genome-wide association study. *Yi Chuan Hered.* 39, 707–716. <https://doi.org/10.16288/j.yczz.16-419>.
- Wang, Z.-T., Tan, C.-C., Tan, L., Yu, J.-T., 2019. Systems biology and gene networks in Alzheimer's disease. *Neurosci. Biobehav. Rev.* 96, 31–44. <https://doi.org/10.1016/j.neubiorev.2018.11.007>.
- Wartman, L.D., 2018. The future of cancer treatment using precision oncogenomics. *Cold Spring Harb. Mol. Case Stud.* 4. <https://doi.org/10.1101/mcs.a002824>.
- Werner, T., 2008. Bioinformatics applications for pathway analysis of microarray data. *Curr. Opin. Biotechnol.* 19, 50–54. <https://doi.org/10.1016/j.copbio.2007.11.005>.
- Wu, M.C., Lin, X., 2009. Prior biological knowledge-based approaches for the analysis of genome-wide expression profiles using gene sets and pathways. *Stat. Methods Med. Res.* 18, 577–593. <https://doi.org/10.1177/0962280209351925>.
- Xiong, H., Choe, Y., 2008. Dynamical pathway analysis. *BMC Syst. Biol.* 2, 9. <https://doi.org/10.1186/1752-0509-2-9>.
- Xu, D., Tian, Y., 2015. A comprehensive survey of clustering algorithms. *Ann. Data Sci.* 2, 165–193. <https://doi.org/10.1007/s40745-015-0040-1>.
- Yu, P., Lin, W., 2016. Single-cell transcriptome study as big data. *Genomics Proteomics Bioinformatics* 14, 21–30. <https://doi.org/10.1016/j.gpb.2016.01.005>.
- Yu, C., Woo, H.J., Yu, X., Oyama, T., Wallqvist, A., Reifman, J., 2017. A strategy for evaluating pathway analysis methods. *BMC Bioinformatics* 18, 453. <https://doi.org/10.1186/s12859-017-1866-7>.
- Yuryev, A., 2008. In silico pathway analysis: the final frontier towards completely rational drug design. *Expert Opin. Drug Discovery* 3, 867–876. <https://doi.org/10.1517/17460441.3.8.867>.
- Yuryev, A., 2011. Integrating fragmented software applications into holistic solutions: focus on drug discovery. *Expert Opin. Drug Discovery* 6, 383–392. <https://doi.org/10.1517/17460441.2011.557659>.
- Zeeberg, B.R., Feng, W., Wang, G., Wang, M.D., Fojo, A.T., Sunshine, M., Narasimhan, S., Kane, D.W., Reinhold, W.C., Lababidi, S., Bussey, K.J., Riss, J., Barrett, J.C., Weinstein, J.N., 2003. GoMiner: a resource for biological interpretation of genomic and proteomic data. *Genome Biol.* 4, R28.
- Zhou, X., Lei, L., Liu, J., Halu, A., Zhang, Y., Li, B., Guo, Z., Liu, G., Sun, C., Loscalzo, J., Sharma, A., Wang, Z., 2018. A systems approach to refine disease taxonomy by integrating phenotypic and molecular networks. *EBioMedicine* 31, 79–91. <https://doi.org/10.1016/j.ebiom.2018.04.002>.
- Zimmermann, M.T., 2018. The importance of biologic knowledge and gene expression context for genomic data interpretation. *Front. Genet.* 9, 670. <https://doi.org/10.3389/fgene.2018.00670>.

# Glossary and index

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**Adherens junctions** Adherens junctions (AJs) are a type of intercellular junctions composed of the transmembrane protein E-cadherin and intracellular components, such as the  $\beta$ - and  $\alpha$ -catenins and plakoglobin. In AJs the cytoplasmic side of E-cadherin is connected to the actin cytoskeleton through catenins. AJs provide maturation, stability, and plasticity of the cell-cell contact.

**Adrenal zona glomerulosa** Adrenal zona glomerulosa is the outermost layer of adrenal cortex that produces the major mineralocorticoid hormone aldosterone.

**Aldosterone** Aldosterone is a key mineralocorticoid hormone that has a crucial role in maintaining the electrolyte and water balance in the body and thus is important for blood pressure regulation.

**Alveolar epithelial cell** The alveolar epithelial cells (pneumocytes) line the alveolar compartment of the lungs. There exist two types of alveolar cells: type I (the prevailing type) and type II. Type I alveolar cells are squamous and extremely thin, and they are involved in the process of gas exchange between the alveoli and the blood. Type II alveolar cells are involved in the secretion of surfactant proteins that line the alveolar lumen.

**Alveolar macrophages** The alveolar macrophages are a type of macrophage found in the pulmonary alveolus, near the alveolar epithelial cells. The alveolar macrophages are cells of the innate immune system, and they remove various infectious or allergic particles from respiratory surfaces. They represent the first line of defense against airborne pathogens.

**Amylin** Amylin is a peptide pancreatic hormone cosecreted with insulin by pancreatic beta cells in response to nutrient stimuli. Amylin promotes satiety and acts as a partner of insulin to lower blood glucose levels.

**Antigen-presenting cells** Antigen-presenting cells (APCs) are a large group of various cell types that trigger the cellular immune response by processing an antigen and exposing it in a form recognizable by T cells in a process known as antigen presentation.

**Apoptosis** Apoptosis is a highly regulated chain of events leading to cell destruction that occurs in multicellular organisms. Apoptosis eliminates damaged or redundant cells, and it is required for normal tissue development and homeostasis.

**Aqueous humor drainage** Aqueous humor (AH) is a transparent liquid that occupies the space between the crystalline lens and the cornea of the eye. AH resembles plasma, but it contains lower protein and glucose concentrations. AH nourishes the cornea and the lens, and it is involved in intraocular pressure maintenance.

**Aqueous humor production and function** The regulation of aqueous humor (AH) outflow is important for intraocular pressure maintenance. The drainage path for AH starts in the posterior chamber of the eye. Further, AH flows into the area between the posterior iris and the anterior lens and then through the pupil to enter the anterior chamber. From the anterior chamber, AH leaves the eye through the trabecular meshwork (TM) and flows into Schlemm's canal. AH then flows through the collector channels into the episcleral veins. The greatest resistance to AH outflow is provided by the TM.

**Aura** Migraine aura refers to a pattern of neurological symptoms that precede the headache. The most common symptoms associated with an aura are temporary visual changes such as blind spots, flashing lights, zig-zagging lines, and double vision. An aura usually develops gradually over a few minutes and lasts for up to an hour.

**Autophagy** Autophagy is a conserved eukaryotic process in which excessive or dysfunctional intracellular components are delivered to lysosomes for degradation. The three major types of autophagy include macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, targeted cytoplasmic constituents are isolated from the rest of the cell within a double-membrane vesicle, the autophagosome.

**Basal epithelial cell** Basal cells are found in the deepest (basal) layer of the epithelium.

**Basement membrane** The basement membrane is a thin protective layer of extracellular matrix that underlies or surrounds epithelial or endothelial cells and separates them from other cells, for example, connective tissue cells.

**Blood pressure** Blood pressure is the pressure exerted by circulating blood on the walls of blood vessels.

**Bone remodeling** Bone remodeling is a dynamic process that maintains bone strength and ion homeostasis by replacing discrete parts of old bone with newly synthesized bone matrix, while bone resorption is performed by large immune cells called osteoclasts; osteoblasts are a type of specialized connective tissue-related cell that is responsible for making new bone. Bone remodeling is impaired in osteopetrosis due to inadequate osteoclast function and the impairment of bone resorption.

**Cadherin** Cadherins are a family of transmembrane calcium-dependent cell-cell adhesion molecules. Cadherins provide stability to cell-cell contacts, and they regulate their formation.

**Calcification** Calcification is an increase in the amount of calcium salts in a tissue.

**Cardiomyocyte** Cardiomyocytes are the principle muscular cells that make up the heart, and they are responsible for generating contractile force.

**Cardiomyocyte hypertrophy** Cardiac hypertrophy is the adaptive enlargement of cardiomyocytes in response to pressure or volume-related stress, which in turn leads to thickening of the heart muscle.

**Caveolae** Caveolae are small invaginations found in the plasma membrane of a variety of cell types and they are involved in signal transduction and membrane trafficking.

**CD4+ T cell** CD4+ T cells are a subtype of T cells (T lymphocytes) that recognize peptides presented on the MHC class II molecules of antigen-presenting cells. CD4+ T cells protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) by stimulating B-cell maturation and the activation of other immune cells.

**CD8+ T cell or killer T cell** Cytotoxic T cells (killer T cells) are a subtype of T cells (T lymphocytes) that eliminate infected or damaged cells. The antigens recognized by cytotoxic T cells typically come from processed cytosolic proteins. Most cytotoxic T cells express T-cell receptors (TCRs), which can recognize a specific antigen. Cytosolic antigens are bound to class I MHC molecules, and in order for the TCR to bind to the class I MHC molecule, the former must be accompanied by a glycoprotein called CD8.

**Cell-cell adhesion** Cell-cell adhesion (intercellular adhesion) is a biological process by which cells form attachments to other cells via specialized cell adhesion molecules. Intercellular adhesion is a fundamental process underlying the formation of multicellular organisms. The major types of cell-cell adhesions include adherens junctions, tight junctions, and desmosomes.

**Chemokines** Chemokines are a family within a larger group of extracellular signaling molecules called cytokines. Chemokines are secreted low-molecular-weight proteins that can induce chemotaxis-directed movement of a cell in response to a molecular stimulus.

**Chemotaxis** Chemotaxis is the directional movement or orientation of cells along a gradient of concentration of a chemical substance.

**Chondrocyte** Chondrocytes are the only cell type present in healthy cartilage tissue. Chondrocytes are responsible for the synthesis and turnover of the cartilaginous ECM, whose main components are collagens and proteoglycans.

**Chondroptosis** Chondroptosis refers to the process of nontypical programmed death (apoptosis) in chondrocytes that involves specific features such as cytoplasmic vacuolization without nuclear fragmentation. Chondroptosis is a highly regulated process required for the cartilage degradation that occurs during skeleton development.

**Cilia** Cilia are thin protuberances (less than 1 μm in width and 3–2 mm in length) on the surface of eukaryotic cells that contain microtubule cytoskeleton structures. Cilia can be multiple or single and motile or nonmotile (primary). Motile cilia are responsible for cell locomotion or the movement of fluids surrounding cells, whereas primary cilia serve as receptor organelles. Cilia are essential for the development and function of certain animal tissues.

**Ciliopathy** Ciliopathies are group of genetic disorders with a wide spectrum of phenotypes caused by mutations in genes encoding ciliary proteins, which in turn affect cilia structure or function.

**Coagulation cascade** The coagulation cascade is a complex set of reactions triggered in response to vascular damage involving platelets and clotting factors, which leads to the formation of a fibrin clot.

**Cochlear hair cell** Cochlear hair cells are the sensory cells of the auditory system. These cells possess stereocilia connected to the tectorial membrane. During auditory stimulation, sound waves in the cochlea cause deflections of the hair cell stereocilia, which create an electrical signal in the hair cell.

**Cochlea** The cochlea is a snail-shaped canal in the osseous labyrinth of the inner ear that contains the sensory organ of hearing termed the organ of Corti.

**Complement system** The complement system is a group of small proteins that “complement” the ability of the antibody system to eliminate cellular pathogens. Proteins of the complement system, produced by the liver and circulating in the blood as inactive precursors, promote inflammation and attack the pathogen’s plasma membrane.

**Corneodesmosomes** Corneodesmosomes are specialized cell-cell adhesion structures that interconnect corneocytes, the major cell type in the stratum corneum. Corneodesmosomes maintain a strong epidermal sheet structure, and their disruption leads to desquamation.

**Cortical spreading depression** Cortical spreading depression (CSD) is a wave of slowly propagating excitation (depolarization) of brain cells followed by the inhibition of neuronal activity. CSD has been implicated in the pathophysiology of migraine.

**C-peptide** The C-peptide is a 31-amino acid long peptide that is a by-product of insulin production. It is used in clinical tests as a marker of insulin production.

**Cyst** A cyst is a pathological closed cavity in a tissue. Cysts have a distinct wall and may contain air, fluids, or semisolid material. Cells forming the wall of a cyst are abnormal compared with the surrounding cells.

**Cytokines** Cytokines are a broad category of small proteins released by immune cells that participate in cell-to-cell communication and in the regulation of immune responses. Cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors.

**Desmosome** Desmosomes are a type of intercellular junction mediated by desmosomal cadherins bound on their intracellular side to the intermediate filament (keratin) cytoskeleton through the cytoplasmic plaque proteins, plakoglobin, and plakophilins, as well as other proteins. Desmosomes help connected cells withstand mechanical forces, and they participate in cell-cell signaling.

**Diabetic ketoacidosis** Diabetic ketoacidosis is a metabolic complication of diabetes characterized by hyperglycemia, decreased serum pH, and an increased serum levels of ketones.

**Diastolic blood pressure** Diastolic pressure is the minimal pressure of circulating blood on the arterial walls that occurs during heart relaxation.

**Drusen formation** Drusen are small yellow or white accumulations of extracellular material in the macula between the Bruch’s membrane and retinal pigment epithelium of the eye, which consist of proteins and lipids. The presence of a few small drusen is normal with advancing age; however, the buildup of larger and more numerous drusen in the macula is a hallmark of age-related macular degeneration (ARMD).

**Dura mater** The dura mater is the outermost and toughest of the three sheaths covering the central nervous system (the brain and spinal cord). Made up of connective tissue, the dura mater contains two layers: the outer layer, which is rich in blood vessels, and the inner layer, which contains large channels known as dural venous sinuses, located between the two layers.

**Effector memory T cells** Effector memory T cells are a subset of antigen-experienced T cells that can be distinguished from another subset of memory T cells, the central memory T cells, by the presence of different protein markers expressed on their surface. Effector memory T cells can enter inflammation sites outside the lymphoid tissues.

**Eicosanoids** Eicosanoids are lipid signaling mediators derived from arachidonic acid and related polyunsaturated fatty acids.

**Endochondral ossification** Endochondral ossification is the process of bone development in which growing cartilage is gradually replaced by bone tissue.

**Endocytosis** Endocytosis is a highly conserved biological process in eukaryotes by which a cell internalizes extracellular substances by engulfing them with its membrane in an energy-dependent manner. The major variations of endocytosis include phagocytosis (ingestion of larger particles) and pinocytosis (ingestion of fluids or macromolecules).

**Endoplasmic reticulum (ER)** The endoplasmic reticulum (ER) is an organelle that forms a continuous network of membrane-enclosed tubules and sacs (cisternae). The ER is involved in protein folding and the transport of newly synthesized proteins to the Golgi apparatus.

**Endoplasmic reticulum stress response** The endoplasmic reticulum protein response (unfolded protein response, UPR) is a highly conserved adaptive process in eukaryotes triggered by a buildup of unfolded and/or misfolded proteins in the endoplasmic reticulum lumen. The UPR leads to the restoration of normal cellular function or the elimination of a severely damaged cells via apoptosis.

**Endothelial cells** Endothelial cells (ECs) comprise a single layer of cells that line the inside of blood and lymph vessels, and they mediate the selective movement of substances and cells between the bloodstream and surrounding cells.

**ERAD** ERAD stands for endoplasmic reticulum-associated protein degradation that is a cellular process that targets misfolded proteins for degradation by the cytoplasmic ubiquitin-proteasome system.

**Erythropoiesis** Erythropoiesis is the process of the formation of red blood cells, which occurs in the bone marrow.

**Estradiol** Estradiol is the major steroid female sex hormone responsible for the maintenance of fertility.

**Excitotoxicity** Excitotoxicity refers to neuronal cell damage or death due to excessive stimulation by neurotransmitters such as glutamate and similar substances.

**Extracellular matrix proteins** The extracellular matrix (ECM), an essential component of most tissues in multicellular organisms, is a noncellular network of macromolecules secreted by surrounding cells. The ECM provides structural support to tissues, and it is strongly involved in intercellular signaling.

**Fatty acid beta oxidation** Beta oxidation of fatty acids is the catabolic process in which the fatty acyl chain is broken down into acetyl-CoA molecules.

**Fibrosis** Fibrosis is the development of excessive fibrous connective tissue and the accumulation of extracellular matrix proteins in an organ or tissue, which occurs as a reparative response to tissue damage. Fibrosis leads to scarring and the thickening of affected tissue and a disruption to its function.

**Gluten and gliadin** Gluten is a general name for the mixture of storage proteins found in the endosperm of cereal grains. Gluten exposure activates an immune response in susceptible patients causing certain health conditions such celiac disease and nonceliac gluten sensitivity. Gluten proteins are classified into two major groups based on their solubility in aqueous alcohol: prolamins and glutelins. Gliadin is a prolamin present in

wheat and other cereal grains of the genus *Triticum*. Gliadin causes bread to rise during baking, and it is thought to be primarily responsible for the negative effects of gluten.

**Glycogenesis** Glycogenesis is the process of glycogen formation from glucose.

**Glycolysis** Glycolysis is the catabolic process that converts glucose to pyruvate.

**Goblet cells** Goblet cells are columnar epithelial cells that secrete gel-forming proteins called mucins (major constituents of mucus). Goblet cells are typically found in the epithelial lining of organs, for example, the respiratory and gastrointestinal tracts, and they are surrounded by stratified squamous cells.

**Hematuria** Hematuria is a condition in which blood is found in the urine. Hematuria can be gross (visible discoloration of the urine) or microscopic (invisible by the naked eye) and can be caused by various problems with the kidneys or the urinary tract.

**Hepatic cirrhosis** Hepatic cirrhosis is a chronic degenerative disease characterized by irreversible replacement of normal liver cells by scar tissue resulting from long-term liver damage.

**Hepatocytes** Hepatocytes are the main cell type of the liver parenchyma, and they occupy approximately 80% of the liver volume. Hepatocytes are responsible for major liver functions including detoxification, protein synthesis, and the metabolism of lipids and carbohydrates.

**HIV latency** HIV latency is characterized by a transcriptionally silent viral genome that retains its ability to reactivate and replicate.

**Homeostasis** Homeostasis is self-regulation, the ability of a system to maintain a stable equilibrium and constancy of its internal state through coordinated reactions.

**Human leukocyte antigen (HLA) complex** The human leukocyte antigen (HLA) complex is the group of genes located on chromosome 6 that encode proteins of the major histocompatibility complex (MHC).

**Hyperandrogenism** Hyperandrogenism is a medical condition characterized by excessive levels of androgens in a female body.

**Hyperglycemia** Hyperglycemia refers to an abnormally high blood sugar level, and it is a hallmark of diabetes.

**Hyperlipidemia** Hyperlipidemia is the condition with excessive levels of triglycerides and cholesterol in the blood, which is usually an asymptomatic state, but it can trigger a number of diseases.

**Hyperseborrhea** Hyperseborrhea is the excessive production of sebum by sebaceous glands. It can cause acne as the thick sebum provides a breeding ground for the bacterium *Propionibacterium acnes*, which participates in acne pathogenesis.

**Hyperplasia** Hyperplasia is a rise in the number of cells in an organ or tissue due to their increased proliferation. Hyperplasia often precedes the development of cancer.

**Hyperproliferation** Hyperproliferation refers to an abnormally high rate of cell proliferation.

**Hypertrophy** Hypertrophy is an increase in size of an organ or tissue caused by an increase in the size of its constituent cells.

**Hypoxia** Hypoxia is an abnormally low oxygen level in a tissue or organ.

**IgE-mediated sensitization** In allergy the immune response starts with allergic sensitization, which can be described as follows. Upon encounter with an allergen, an antigen-presenting cell (APC) presents the allergen to T cells that are then induced to differentiate into Th-2 cells. Those cells in turn promote the differentiation of B cells into allergen-specific immunoglobulin E antibody (IgE) producing plasma cells. Further the allergen-specific IgE binds to the surface of mast cells and basophils, which can then recognize the allergen because they are sensitized to the allergen. The next time the allergen enters the body, it can bind to the IgE molecules on the surface of the mast cells and basophils, and an allergic reaction to the allergen might develop.

**Immunoglobulin E antibodies** Immunoglobulin E (IgE) antibodies are a type of antibodies produced by plasma B cells in response to allergens and parasites. IgE monomers consist of two heavy chains and two light chains. IgE antibodies have an essential role in the allergic immune response.

**Inflammasome** The inflammasome is a multiprotein complex of the innate immune response. The inflammasome activates the expression of the proinflammatory interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-18 (IL18) and promotes inflammation. Dysregulation of inflammasome function is involved in the pathogenesis in a variety of autoimmune diseases.

**Inner ear** The inner ear is the innermost portion of the ear that contains organs responsible for hearing and for the sense of balance. Located in the temporal bone, the inner ear has three essential parts, the cochlea, vestibule, and semicircular canals.

**Insulin** Insulin is a peptide anabolic hormone that regulates glucose homeostasis by allowing glucose entry into cells and its conversion into the storage molecule glycogen, which is metabolized during an energy deficiency.

**Insulin resistance** Insulin resistance is a pathological condition characterized by the impaired ability of insulin target tissues to respond to insulin. Insulin resistance leads to an excess concentration of insulin in blood (hyperinsulinemia) as pancreatic beta cells produce more insulin in response to the hyperglycemia caused by the inability of the cells to respond to insulin.

**Interleukins** Interleukins are a subgroup of a larger group of extracellular signaling molecules called cytokines. Interleukins are low-molecular-weight proteins involved in the functioning of both the adaptive and innate components of the immune system.

**Interstitial edema** Interstitial edema is a condition of abnormally large interstitial fluid (IF) volume. IF is a solution that fills the spaces between cells within tissues (interstitial spaces).

**Intracellular sorbitol accumulation** In diabetes the intracellular accumulation of the sugar alcohol sorbitol leads to changes in crystalline structure and, thus, accelerates cataract development.

**Intraflagellar transport** Intraflagellar transport is a specialized intracellular process in eukaryotes, which is essential for the biogenesis of cilia. It is the bidirectional transport of structural and functional ciliary components along microtubules to the tip of the cilium and back to the cell body.

**Intraocular pressure** Intraocular pressure (IOP) is the intraocular fluid pressure inside the eyeball. IOP depends on the balance between the production and drainage of aqueous humor mainly through the trabecular meshwork. IOP is increased in glaucoma.

**Ischemia** Ischemia is the restricted blood supply to a tissue or organ caused by an obstruction or narrowing of a blood vessel.

**Islet alpha cells** Islet alpha cells are one of the major cell types in the pancreatic Langerhans islands. When blood glucose and insulin levels decrease, alpha cells produce and secrete glucagon that regulates glycemia by stimulating glycogenolysis and gluconeogenesis and by blocking glycolysis in hepatocytes.

**Islet beta cells** Islet beta cells are the major cell type in the pancreatic Langerhans islands. Their main function is to synthesize and secrete insulin in response to elevated blood glucose levels.

**Lamellar granules** Lamellar granules (also called lamellar bodies, keratinosomes, Odland bodies, and membrane-coating granules MCGs) are membrane-bound vesicles produced by skin keratinocytes or alveolar cells of the lung. In the skin, lamellar granules are secreted into the extracellular space between the epidermal layers, and they contain the molecules, lipids, and proteins required for maintaining the lipid barrier and the layered structure of the epidermis. In the lungs, lamellar bodies participate in the production of pulmonary surfactant.

**Langerhans cells** Langerhans cells (LCs) are antigen-presenting cells (dendritic cells) of the epidermis. LCs contain a specific type of organelle known as Birbeck granules found exclusively in these cells.

**Lens epithelial cells** The lens epithelium located in the anterior surface of the lens is composed of cuboidal-shaped epithelial cells, which regulate lens homeostasis.

**Lipid skin barrier** The lipid skin barrier is a layer of lipids, ceramides, and fatty acids produced by keratinocytes in the stratum corneum layer of epidermis. The lipid matrix prevents excessive water loss through the epidermis, and it forms a physical barrier against harmful agents.

**Lipofuscin aggregation** The incomplete degradation of proteins inside lysosomes leads to the accumulation of granules containing the insoluble autofluorescent pigment lipofuscin. Lipofuscin inhibits the intracellular proteasomal- and lysosomal-autophagic systems.

**Major histocompatibility complex (MHC) class II** The major histocompatibility complex (MHC) class II is a heterodimeric protein complex on the surface of antigen-presenting cells. MHC class II molecules have a fundamental role in processing extracellular antigens and presenting them to T cells.

**Major histocompatibility complex (MHC) class I** The major histocompatibility complex (MHC) class I is a heterodimeric protein complex on the surface of many cell types. The MHC class I molecules have a fundamental role in processing extracellular antigens and presenting them to CD8+ T cells.

**Matrix metalloproteinases** Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases. These enzymes are responsible for processing and degrading most of the constituents of the extracellular matrix. Their targets include a variety of extracellular proteins and other molecules, and, thus, MMPs can be viewed as regulators of key cellular and tissue processes.

**Mechanoelectrical transducer channel** The mechanoelectrical transducer (MET) channels are ion channels on the tips of stereocilia. The deflection of stereocilia provokes the mechanical opening of these channels and the entrance of cations, which generate action potentials.

**Mesangial cells** Mesangial cells are contractile cells in the kidneys that make up the mesangium of the glomeruli. The primary function of mesangial cells is to remove trapped residues and aggregated proteins from the glomerular basement membrane, thus keeping the filter free of debris.

**Middle ear** The middle ear is the internal part of the ear that conducts sound from the outer to the inner ear.

**Motor neuron** A motor neuron is an efferent neuron (transmitting the impulse outside the brain or spinal cord) located in the spinal cord whose axon projects outside the spinal cord (lower motor neuron) or in the motor cortex whose axon descends to the spinal cord (upper motor neurons). Motor neuron axons conduct signals to the effectors, mainly muscles or lower motor neurons, to produce effects.

**Mucociliary clearance** Mucociliary clearance (MCC) is one of the major defense mechanisms of the lung in which mucus and potentially harmful foreign substances contained in it are moved out of the lung. Cilia on the surface of airway epithelial cells provide the force necessary for mucus movement.

**Mucus** Mucus is a heterogeneous mixture of secreted polypeptides (termed mucins), cells, and cellular debris that may be tethered together at the fluid surface by oligomeric mucin protein complexes.

**Naïve T cells** Naïve T cells are mature T cells in the bone marrow that have gone through the process of positive and negative selection and can respond to newly recognized pathogens.

**Necrosis** Necrosis is the premature death of living cells by autolysis that is caused by disease, trauma, or insufficient blood supply to the organ or tissue.

**NOD-like receptors (NLRs)** The NOD-like receptors (nucleotide-binding oligomerization domain-like receptors, NLRs) are cytoplasmic pattern recognition receptors. NLRs can bind to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) inside cells, and they have a variety of functions in the regulation of inflammatory and apoptotic responses. The NLR family consists of several proteins divided into subfamilies based on their N-terminal protein-interacting domains.

**Nuclear pore complex** Nuclear pore complexes are openings in the nuclear envelope that allow for the highly selective transport of various substances into and out of the nucleus.

**Optic nerve** The optic nerve (cranial nerve II, CN II) is a paired nerve that conducts visual impulses from the retina to the brain. The optic nerve consists of retinal ganglion cell axons and glial cells. In glaucoma the optic nerve is damaged due to increased intraocular pressure.

**Organ of Corti** The organ of Corti is the auditory organ situated in the cochlea of the inner ear. The sensory hair cells that make up the organ of Corti are responsible for the transduction of auditory impulses into neural signals.

**Osmolarity** Osmolarity or osmotic concentration is the solute concentration defined as the number of osmoles of solute per liter of solution.

**Osteoblast** An osteoblast is a specialized connective tissue-related bone cell responsible for the synthesis and mineralization of bone during both the initial bone formation and bone remodeling.

**Osteoclast** Osteoclasts are the giant multinuclear bone cells of hematopoietic origin responsible for the dissolution and absorption of bone. This function is critical for the maintenance, repair, and remodeling of bones.

**Ovarian cyst** An ovarian cyst is a fluid-filled closed cavity inside the ovary. Ovarian cysts are often asymptomatic; however, they may produce abdominal or back pain and disrupt the menstrual cycle.

**Ovarian follicle** The ovarian follicle is a spherical structure inside the ovaries that contain an egg (ovum, egg cell, or oocyte). Ovarian follicles also contain granulosa (or follicular) cells that surround the oocyte and theca cells and secrete hormones, which effect the menstrual cycle.

**Oxidative stress** Oxidative stress occurs when the antioxidant defense system is unable to neutralize the harmful effects of reactive oxygen species (ROS).

**Paneth cell** Paneth cells are specialized secretory epithelial cells of the small intestine that produce antimicrobial peptides, and they are key players in the intestinal innate immune defense.

**Pathogen pattern recognition** During the initial stages of the response to infection, the innate defense system employs pattern recognition receptors (PRRs) that recognize components of invading pathogens. The PRRs are able to detect the evolutionarily conserved molecular structures called pathogen-associated molecular patterns (PAMPs) derived from bacteria and viruses and initiate an antimicrobial inflammatory response. At the same time, PRRs can also detect damage-associated molecular patterns (DAMPs) released from host cells during cell damage or death and initiate a noninfectious inflammatory response.

**Peyer's patches** Peyer's patches (PPs) are organized lymphoid nodules in the mucous layer of the ileum (a segment of the small intestine). PPAs appear as round or oval aggregates located in the epithelial mucosal membrane lining, and they have a primary role in the induction of mucosal immunity in the gut.

**Phagocytosis** Phagocytosis is a form of endocytosis by which a cell internalizes large ( $>0.5\text{ }\mu\text{m}$ ) particles via the formation of an internal compartment known as a phagosome, which is further fused with a lysosome for degradation. Professional immune cells (macrophages, neutrophils, and others) employ phagocytosis to remove invading pathogens.

**Phagosome (phagocytosis)** Phagosomes are cytoplasmic membrane-bound vesicles that contain particles that were internalized via phagocytosis.

**Platelet** Platelets are small circulating nonnucleated fragments derived from megakaryocytes in the bone marrow with a key role in hemostasis (the process that stops bleeding from a ruptured vessel) and thrombosis (the formation of a clot within a blood vessel obstructing the blood flow).

**Pleiotropic gene** A pleiotropic gene is one that when mutated produces several markedly different phenotypic traits.

**Podocytes** Podocytes are highly specialized epithelial cells in the visceral layer of the kidney's Bowman capsule (glomerulus) attached to the basement membrane of nearby capillaries via cytoplasmic pedicles (foot-like projections). Podocytes participate in the formation of the glomerular filtration barrier.

**Prediabetes** Prediabetes is an intermediate condition with glycemic numbers above normal but below the diagnostic range for diabetes.

**Progesterone** Progesterone is an essential steroid sex hormone involved in the regulation of the menstrual cycle, early pregnancy support, and embryogenesis.

**Proinflammatory cytokines** Cytokines are a broad category of small proteins released by immune cells that participate in cell-to-cell communication and that regulate immune responses. The proinflammatory cytokines (interleukins, tumor necrosis factor (TNF), interferon gamma (IFN-gamma), granulocyte-macrophage colony stimulating factor (GMCS-F), and others) are secreted primarily by macrophages and T- helper cells, and they upregulate proinflammatory reactions.

**Protein aggregation** Protein aggregation is a biological process in which proteins with abnormal secondary or tertiary structure accumulate and stick together forming organized aggregates. The aggregation process is associated with a variety of health conditions including many neurodegenerative diseases.

**Proteinuria** Proteinuria is the presence of larger than normal amounts of protein in the urine, and it can be a sign of disease.

**Provirus** The provirus forms when viral DNA becomes integrated into the host genome.

**Pulmonary artery smooth muscle cells (PASMCs)** Pulmonary artery smooth muscle cells (PASMCs) are nonstriated muscle cells of the pulmonary artery wall localized in the arterial media layer (middle coat of the artery).

**Pulmonary emphysema** Pulmonary emphysema is a long-term lung illness characterized by decreased respiratory function and shortness of breath caused by irreversible pathological changes of alveoli at the end of the bronchioles and the destruction of alveolar elastic tissue. Pulmonary emphysema is a one of the conditions described as chronic obstructive pulmonary disease (COPD).

**Renal tubular acidosis** Renal tubular acidosis is a medical condition characterized by metabolic acidosis that occurs due to defective renal acid excretion.

**Retinal pigment epithelial cells** Retinal pigment epithelial (RPE) cells comprise a single layer of specialized pigmented cells located between the retinal photoreceptor (PR) cells and the choroid. RPE cells are essential for the maintenance of the retina, and their functions include nourishment of the PR cells, absorption of excessive light, reisomerization and storage of the retinoid, and phagocytosis of shed PR membranes. RPE cells derive from the ectoderm and are considered part of the retina.

**Retinoic acid** Retinoic acid is a vitamin A (retinol) derivative involved in the regulation of cell differentiation and embryonic development.

**Ribbon synapses** A ribbon synapse is a neuronal synapse structurally different from other synapses by the presence of an electron-dense structure called the synaptic ribbon, which helps to keep synaptic vesicles near the active zone. Ribbon synapses are found in various sensory receptor cells, for example, auditory hair cells of the cochlea, and they are characterized by increased performance of the synapse.

**ROS** Reactive oxygen species (ROS) are chemically reactive oxygen containing molecules commonly produced during normal metabolic processes involving oxygen. ROS can damage all essential cellular components including lipids, proteins, and DNA.

**Sarcomere** The sarcomere is the contractile unit of striated muscle myofibrils that consists of a large number of parallel actin (thin) and myosin (thick) protein filaments. In the sarcomere, actin filaments are tethered at their plus ends to structures located at the lateral ends of each sarcomere called Z discs, and myosin is bound to the M line in the middle of the sarcomere. Additional proteins, such as nebulin and titin, are involved in maintaining sarcomere structure and stability.

**Sebocyte** Sebocytes are cells that constitute sebaceous glands and produce sebum (a mixture of fatty acids and other molecules). Sebocytes participate in pathological processes that take place in the sebaceous gland, for example, acne.

**Smooth muscle cells of blood vessels** Smooth muscle cells (SMC) of blood vessels are non-striated muscle cells found in the middle layer of the vascular wall.

**Stereocilia** Stereocilia are thin projections on cochlear hair cells that respond to fluid motion and are involved in mechanosensing. Despite a similar name, stereocilia are different from cilia (microtubule cytoskeleton-based structures), and they contain actin cytoskeleton, similarly to microvilli.

**Stratum corneum** The stratum corneum is the outer layer of the epidermis composed of dead corneocytes filled with keratin (skin cells) submerged in an intercellular matrix composed of lipids and fatty acids. The stratum corneum serves as a tough protective barrier for the inner layers of living cells.

**Stratum granulosum** The stratum granulosum (granular layer) is one of the intermediate layers of the epidermis localized between the stratum corneum and stratum spinosum (although in thick skin, there is an additional layer just underneath the stratum corneum called the stratum lucidum). Granular layer keratinocytes contain dense lipid-rich granules called keratohyalin granules, which participate in the formation of the hydrophobic barrier in the skin.

**Synapse** The synapse is a specialized connection between two neurons or between a neuron and an effector cell where a nerve impulse can be conducted between the two cells.

**Synaptic cleft** The synaptic cleft is the space between the neuron and its target cell, and it is where the release of neurotransmitters occurs.

**Systolic blood pressure** Systolic blood pressure is the pressure of circulating blood on the arterial walls at the moment of heart muscle contraction.

**Tectorial membrane** The tectorial membrane is a band of extracellular matrix in the cochlea located above the inner and outer hair cells of the organ of Corti. The tectorial membrane is connected to stereocilia of the outer hair cells, and it participates in the mechanotransduction of sound. During auditory stimulation the tectorial membrane directly stimulates the outer hair cells and creates liquid movements that stimulate the inner hair cells.

**Th17 cells** Th17 cells are a subset of T-helper cells that preferentially express the interleukins 17A, 17F, 21, and 22. Th17 cells act as proinflammatory agents by recruiting neutrophils and macrophages to the infected site. Th17 cells have been implicated in the development of autoimmune diseases.

**Tissue resident T cells** Tissue resident T cells are a subset of T cells that reside in tissues without recirculating, and they provide enhanced protection against infections that enter through body surfaces.

**Toll-like receptors** Toll-like receptors belong to a family of membrane proteins that can directly bind microbial molecules or proteins to initiate the innate immune response.

**Trabecular meshwork** The trabecular meshwork (TM) is an area in the anterior chamber of the eye lined by cells called trabeculocytes. The TM provides resistance to aqueous humor flow, and it is crucial for the maintenance of normal intraocular pressure.

**Type 1 and type 2 T-helper cells** Type 1 and type 2 T-helper cell (Th1, Th2 cell) are T cells that protect against intracellular bacteria and protozoa (Th1) and extracellular parasites (Th2) by stimulating B-cell maturation and by activating other immune cells.

**Usher syndrome** Usher syndrome is a rare genetic disorder characterized by hearing loss and gradual vision loss due to retinitis pigmentosa (progressive degeneration of photoreceptor cells in the retina). The disease is clinically and genetically heterogeneous with at least 15 chromosomal loci being assigned to three clinical Usher syndrome types, namely, USH1A-G, USH2A-C, and USH3A.

**Vasoconstriction** Vasoconstriction is the narrowing of blood vessels caused by the contraction of smooth muscles in their walls. Vasoconstriction decreases blood flow through the vessels and increases blood pressure.

**Vasodilation** Vasodilation is the widening of blood vessels caused by the relaxation of smooth muscle cells in their walls. Vasodilation increases blood flow through the vessels and decreases blood pressure.

**Viral genome encapsidation** Viral genome encapsidation is the process of enclosure of viral DNA or RNA into a protein capsid.

**Viral replication** Viral replication is the production of new viruses inside infected host cells.

**Viral episomal form** Episomal form of a virus refers to a viral latency state in which the virus does not integrate into the host genome, but rather, it exists as an extrachromosomal episome inside the host nucleus.

**Viral synapse** Viral synapse is the cell-to-cell contact between infected and noninfected cells that facilitates the transmission of the virus.

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# Links

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The book combines different domains of knowledge as we refer to medicine, genetics, molecular biology, biochemistry, and system biology. There are comments in all of the chapters on many of the objects and names of specific terms without an exhaustive description. The glossary of terms partially addresses this issue. However, there are many public biomedicine databases which we encourage the reader to explore to better understand the roles and functions of proteins, mutated genes, canonical signaling pathways, or medications.

Here is a short list of comprehensive online resources.

Resources that have been developed by the two large publicly supported research centers, NCBI and EMBL-EBI, comprise the core of all available public databases:

US National Center for Biotechnology Information (NCBI)—develops, distributes, supports, and coordinates access to a variety of biomedical databases and is available at <https://www.ncbi.nlm.nih.gov>.

The European Molecular Biology Laboratory (EMBL)—maintains freely available and up-to-date molecular data resources, and it is available at <https://www.ebi.ac.uk/services>.

The disease pathways that were reconstructed for the book with the help of Pathway Studio software can be browsed or downloaded for analysis (<http://www.transgene.ru/disease-pathways/> or <https://www.smartbio.ai/nbs/pathways>). The complete Elsevier Pathway Collection 2018 contains 2411 manually created pathways which are commercially available at <http://www.pathwaystudio.com>. For public access to selected data from Pathway Studio, please, visit Ask Pathway Studio at <https://mammalcedfx.pathwaystudio.com/app/search>.

To explore protein- or gene-related information, type the name of the gene or protein of interest in these databases

NCBI Gene—a database with gene sequences and functional information, <https://www.ncbi.nlm.nih.gov/gene>;

EMBL UniProt—contains protein sequences and functional information, <https://www.uniprot.org>;

*GeneCards* (Weizmann Inst.)—integrates human gene-centric data from 125 resources, <http://www.genecards.org>;

*The Gene Ontology (GO)*—knowledge of how genes encode biological functions at the molecular, cellular, tissue, and system levels, <http://www.geneontology.org>, <http://amigo.geneontology.org>.

### To explore the genetics of diseases, search the name of the disease or gene in

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*Online Mendelian Inheritance in Man (OMIM)*—a catalog of human genes and genetic disorders, <https://www.omim.org>;

*Genetics Home Reference*—information on the effects of genetic variation on human health, <https://ghr.nlm.nih.gov>;

*NCBI ClinVar*—human variations and phenotypes, <https://www.ncbi.nlm.nih.gov/clinvar/intro>;

*PharmGKB*—pharmacogenomics knowledge resource that collects clinically actionable gene–drug associations and genotype–phenotype relationships, <https://www.pharmgkb.org>;

*OpenTargets*—to explore variant–gene–trait associations from the UK Biobank and GWAS Catalog, <https://genetics.opentargets.org>;

*Orphanet*—the portal of rare diseases, <https://www.orpha.net>;

*GWAS Catalog* (NHGRI-EBI Catalog, <https://www.ebi.ac.uk/gwas>);

International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10), <https://icd.who.int/browse10/2016/en>, <https://www.icd10data.com>. ICD-11, <https://www.who.int/classifications/icd/revision>, <https://icd.who.int>.

### Resources with freely available signaling pathways in a different format

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*Pathway Commons*—integrates pathways from 26 resources in one format (BioPAX), <https://www.pathwaycommons.org>;

*ConsensusPathDB-human*—integrates interaction networks in *Homo sapiens* currently from 32 public resources, <http://cpdb.molgen.mpg.de>;

*WikiPathways*—2763 pathways. WikiPathways is an open, collaborative platform dedicated to the curation of biological pathways, <https://en.wikipedia.org/wiki/WikiPathways>;

*Reactome*—2256 maps for *Homo sapiens*. Reactome is an open-source, open-access, manually curated, and peer-reviewed pathway database, <https://reactome.org>;

*Kyoto Encyclopedia of Genes and Genomes (KEGG)*—530 pathways. KEGG is a public database with a collection of manually drawn pathway maps

representing our knowledge of the molecular interaction, reaction, and relationship networks, <http://www.genome.jp/kegg/pathway.html>;

*Biocarta*—more than 254 pathways, [https://cgap.nci.nih.gov/Pathways/BioCarta\\_Pathways](https://cgap.nci.nih.gov/Pathways/BioCarta_Pathways);

*BioCyc*—a database of computationally predicted metabolic pathways and chemical reactions, <http://www.biocyc.org>;

*PharmGKB*—135 pathways. PharmGKB provides evidence-based diagrams depicting the pharmacokinetics (PK) and/or pharmacodynamics (PD) of drugs, <https://www.pharmgkb.org/pathways>;

*Therapeutically Relevant Multiple Pathways (disease) Database*—97 pathways. TRMPD includes pathways about targeted disease conditions and the corresponding drugs directed at each of these targets, [http://bidd.nus.edu.sg/group/trmp/trmp\\_ns.asp](http://bidd.nus.edu.sg/group/trmp/trmp_ns.asp);

*AOPs knowledgebase stores information about Adverse Outcome Pathways* (<https://aopkb.oecd.org>, <https://aopwiki.org>);

*STRING*—18,838 proteins with network connections for *Homo sapiens* and other organisms. STRING is a database with protein–protein interactions for many species, <https://string-db.org>;

*BioGRID*—23,291 human genes and their interactions. There are 1,664,026 protein and genetic interactions in total, <https://thebiogrid.org>;

*SMPDB* (The Small Molecule Pathway Database)—contains human-specific information about 1451 proteins and 691 interactions, and it claims to contain more than 40,000 “pathways,” <http://smpdb.ca>;

*Cell Signaling*—60 canonical pathways. Cell Signaling is a private company whose website contains interactive signaling pathway diagrams and research overviews, and it is relevant to the pathway antibody products it sells, <https://www.cellsignal.com/contents/science/cst-pathways/science-pathways>;

*Protein Lounge*—789 images of signaling pathways with descriptions. Protein Lounge is a commercial resource with a big collection of poster-like images of metabolic and signaling pathways, <http://www.proteinlounge.com/Pathway/Pathways.aspx>;

*Qiagen/Ingenuity*—more than 500 pathways are available. Ingenuity is a commercial software and database resource with public access to selected data, <https://www.qiagen.com/us/shop/genes-and-pathways/pathway-central/>, <https://targetexplorer.ingenuity.com/index.htm>.

### Also, other databases can be useful

*Human Protein Atlas*—distribution of the proteins across all major tissues and organs in the human body, <https://www.proteinatlas.org>;

*Encodeproject*—encyclopedia of DNA elements, <https://www.encodeproject.org>;

*ClinicalTrials*—a database about clinical studies, <https://clinicaltrials.gov>;  
*DrugBank*—a database that combines comprehensive drug target-related information, <https://www.drugbank.ca>;

*PathGuide*—lists pathway databases and bioinformatics resources, <http://www.pathguide.org>;

*ClinGen*—a National Institutes of Health (NIH)–funded resource that defines the clinical relevance of genes and gene variants for use in precision medicine and research, <https://www.clinicalgenome.org/>;

*CLINVITAE*—a database of clinically observed genetic variants aggregated from public sources. It is operated and made freely available by INVITAE, <http://clinvitae.invitae.com>.

## Other tools and software mentioned in the book

### Commercial

*Ingenuity Pathway Analysis*, <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>;

*Genomatix Software Suite* (Genomatix), <https://www.genomatix.de>;

*MetaCore* (Cortellis), <https://clarivate.com>;

*iPathwayGuide* (Advaita Corporation), <https://www.advaitabio.com>;

*omicX*, <https://omictools.com>.

### Publicly available

*PathVisio*, which is integrated with the WikiPathways database, is a software for pathway reconstruction, visualization, and some types of pathway analysis, <https://www.pathvisio.org>;

*DAVID* is a favorite tool for performing overrepresentation or enrichment analysis (ORA), <https://david.ncifcrf.gov/>;

*Cytoscape* is one of the most frequently used publicly available tools today that can handle all bioinformatic tasks related to pathways, networks, and gene sets, <https://cytoscape.org>;

*GSEA* is integrated with the Molecular Signatures Database (MSigDB), and it is a standard tool for pathway analysis, <http://software.broadinstitute.org/gsea/index.jsp>;

*EnrichNet* is a web application that complements classical overlap-based enrichment analyses (ORA), <http://www.enrichnet.org>/;

*PCxN* finds correlation relationships among multiple pathways/gene sets identified by GSEA, <http://pcxn.org:8080>;

*Bubble GUM* allows the user to automatically extract phenotype signatures based on transcriptomic data using GSEA, <http://www.ciml.univ-mrs.fr/applications/BubbleGUM/index.html>;

*CrossTalkZ* is a statistical tool to assess crosstalk enrichment between node groupings in a network, <https://github.com/uzbit/CrossTalkZ>;

*NetVenn* allows the user to easily compare lists of genes by placing them on the interactome network, <https://wheat.pw.usda.gov/NetVenn/>;

*BinoX* is based on a Monte Carlo approach and designed to identify significant relationships between and within sets of genes, <https://bitbucket.org/sonnhammergroup/binox/wiki/Home>;

*GoMiner* leverages the Gene Ontology (GO) to identify the biological processes, functions, and components represented in experimental gene lists, <https://discover.nci.nih.gov/gominer/index.jsp>.

## Mathematical modeling tools

*CompuCell3D*, <http://www.compuccell3d.org>;

*Chaste*, <http://www.cs.ox.ac.uk/chaste>;

*Virtual Cell*, <http://vcell.org>;

*NetLogo* (<https://ccl.northwestern.edu/netlogo>) and *StarLogo*, (<https://education.mit.edu/project/starlogo-tng>) are the most widely used frameworks to create agent-based models and complex systems in general.

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# DISEASE PATHWAYS

## *An Atlas of Human Disease Signaling Pathways*

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***Disease Pathways: An Atlas of Human Disease Signaling Pathways*** is designed to fill a void of illustrated reviews about the cellular mechanisms of human diseases. It covers 42 of the most common non-oncologic diseases and illustrates the connections between the molecular causes of the disease and its symptoms. This resource provides readers with detailed information about the disease molecular pathways, while keeping the presentation simple.

Pathway models that aggregate the knowledge about protein–protein interactions have become indispensable tools in many areas of molecular biology, pharmacology, and medicine. In addition to disease pathways, the book includes a comprehensive overview of molecular signaling biology and application of pathway models in the analysis of big data for drug discovery and personalized medicine.

This is a must-have reference for general biologists, biochemists, students, medical workers, and everyone interested in the cellular and molecular mechanisms of human disease.

### Key Features

- More than 145 full-color illustrations of the molecular and cellular cascades underlying the disease pathology.
- Disease pathways are based on computational models from Elsevier's Disease Pathway Collection, published for the first time outside of Pathway Studio® commercial software.
- Each relationship on the pathway models is supported by references to scientific articles and can be examined at freely available online resources.



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ISBN 978-0-12-817086-1



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